Zentrum für Molekulare Neurobiologie Universität Hamburg

ZMNH

Report 2002-2004

Front Cover: Mouse hippocampal neuron cultured for 12 days *in vitro*. The culture was transfected with a mammalian expression construct encoding green fluorescent protein to visualize both the axonal and somatodendritic domains.

Foto: Nadia Tagnaouti, PD Dr. Matthias Kneussel

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Vorwort

Das Zentrum für Molekulare Neurobiologie ist ein Forschungszentrum der Universität Hamburg am Fachbereich Medizin. Es betreibt Grundlagenforschung auf dem Gebiet der molekularen Neurobiologie und angrenzender Gebiete. Die gewonnenen Erkenntnisse werden oft schnell auf medizinische und humangenetische Fragestellungen übertragen, wobei entsprechende Mausmodelle von zentraler Bedeutung sind. Transgene und "knock-out" Mäuse, bei denen die Aktivität einzelner Gene spezifisch verändert wird, ermöglichen den Schritt vom Gen zur Physiologie und zur Krankheit, wobei die Erzeugung und Charakterisierung von entsprechenden Mausmodellen eine prominente Rolle spielen. Transgene und „knock-out" Mauslinien, bei denen spezifisch die Aktivität einzelner Gene bzw. Genprodukte verändert wird, haben das Potenzial, Genotyp und (patho)physiologischen Phänotyp miteinander kausal zu verbinden. Neben seiner Hauptaufgabe, der Forschung, ist das ZMNH auch in der Lehre aktiv. Dies geschieht vor allem im Aufbaustudiengang Molekularbiologie, der vom ZMNH geleitet wird (Sprecherin: Schachner).

Den Kern des ZMNH bilden vier von Professoren (Jentsch, Pongs, Schachner, Schaller) geleitete Institute sowie mehrere von Nachwuchswissenschaftlern geleitete, zeitlich befristete Forschergruppen. Hinzu kommt das Institut des Gründungsdirektors Prof. Dr. D. Richter. Die Institute und Gruppen des ZMNH werden durch wissenschaftliche Serviceeinheiten unterstützt. Eine weitgehend eigenständige Verwaltung stellt die notwendige Effizienz und Flexibilität der administrativen Vorgänge sicher, und ein wissenschaftlicher Beirat evaluiert und berät das ZMNH.

Die unabhängigen jungen Forschergruppen sind zentral für das Konzept des Zentrums. Seit 2002 werden von der Stadt Hamburg nunmehr vier Forschergruppen (Kneussel, Riethmacher, Sander/Hoppe, Schimmang) finanziert. Zwei weitere Forschergruppen werden im Rahmen des Heisenbergprogramms (Bach) bzw. des Sonderforschungsbereichs (SFB) 444 (Kornau) finanziert, so dass zur Zeit sechs unabhängige Nachwuchsgruppen am ZMNH arbeiten. Im Verlauf der Berichtsperiode hat Maike Sander einen Ruf als Assistent Professor in der Abteilung für Entwicklungs- und Zellbiologie an der Universität von Kalifornien, Irvine, USA, angenommen und Dietmar Kuhl einen Ruf auf den C4-Lehrstuhl für Molekulare Neurobiologie an der Freien Universität Berlin. Dies unterstreicht den nachhaltigen Erfolg des Konzepts, selbstständiges wissenschaftliches Arbeiten junger Wissenschaftler zu fördern. Dieser Erfolg hat einige Institute des ZMNH (Pongs, Richter, Schaller) dazu bewogen, intern Ressourcen zur Förderung weiterer Nachwuchsforschergruppen, die den jeweiligen Instituten angegliedert sind, zur Verfügung zu stellen.

Sechzehn Jahre nach seiner Gründung (im Jahr 1988) ist das ZMNH inzwischen fest in die Hamburger Wissenschaftslandschaft integriert. Dies äußert sich in einer großen und stetig zunehmenden Zahl wissenschaftlicher Kollaborationen, insbesondere mit Gruppen der Universitätsklinik Eppendorf (UKE). Die diversen und manchmal unerwarteten Phänotypen neuer am ZMNH entwickelter Mauslinien erfordern des Öfteren spezifische Expertisen, die am UKE hervorragend vertreten sind.

Die lokale Vernetzung schlägt sich auch in formalen Forschungsverbünden nieder, in denen das ZMNH federführend beteiligt ist. Genannt seien der am ZMNH

zentrierte Sonderforschungsbereich (SFB) 444 (*Grundlagen neuraler Kommunikation und Signalverarbeitung*; Sprecher: Jentsch), das ebenfalls am ZMNH konzentrierte Graduiertenkolleg 255 (*Neurale Signaltransduktion und deren pathologische Störungen*; Sprecher: Schachner), der SFB 470 (*Glycostrukturen in Biosystemen - Darstellung und Wirkung*; Sprecher: Thiem), die Forschergruppe RNA Transport (Sprecher: Richter) sowie das im Rahmen des vom BMBF initiierten *‚Nationalen Genomforschungsnetzes'* geförderte Projekt zur Pathologie von Erkrankungen des Nervensystems. Die Hamburger Gruppierung (Sprecher: Jentsch) schließt neben fünf ZMNH-Gruppen die Humangenetik des UKE ein.

Forschung am ZMNH

Das ZMNH hat sich als eines der führenden Forschungszentren auf dem Gebiet der molekularen Neurobiologie etabliert. Neben seinen Publikationen - dem Hauptindikator wissenschaftlicher Leistung - schlägt sich dies auch in dieser Berichtsperiode wieder in einer Anzahl angesehener wissenschaftlicher Preise, insbesondere auch an die jüngeren Wissenschaftler, nieder. Zu erwähnen sind hier u.a. die Berliner Professur an der Yale-Universität, der Adolf-Fick-Preis, der Homer W. Smith Award und die Gottschalk-Vorlesung an Thomas Jentsch, der Bickel-Preis der Deutschen Kardiologischen Gesellschaft an Dirk Isbrandt, der Research Award of the European Society of Anaesthesiologists an Patrick Friederich, der Finkelstein-Preis der Norddeutschen Gesellschaft für Pädiatrie an Axel Neu.

Die Gruppen des ZMNH beschäftigen sich primär mit Problemen der molekularen Neurobiologie. Schwerpunkte bilden die Funktion und Bedeutung von Ionenkanälen, die neuronale Entwicklung und Zelldifferenzierung sowie Adhäsionsmoleküle und synaptische Plastizität. Neben diesen rein neurobiologischen Fragestellungen erstreckt sich die Forschung auch auf andere Gebiete der Zell- und Entwicklungsbiologie sowie der (Patho-)Physiologie. So erforschen Wissenschaftler des ZMNH sehr erfolgreich u.a. auch die Entwicklung des Polypen Hydra, die Entwicklung des Pankreas, Bluthochdruck und Herzrhythmusstörungen, Taubheit, Infertilität, Nierenerkrankungen, mentale Retardierung, Polyneuropathien und Regeneration des adulten Nervensystems. Als besonders effizient erweist sich das Zentrum bei der Herstellung und Analyse von "knock-out" Mäusen und herausragende Durchbrüche gelangen in den vergangenen Jahren bei der molekularen Aufklärung monogener menschlicher Erbkrankheiten.

Das Institut für Molekulare Neuropathobiologie (Jentsch) untersucht Fragestellungen des Ionentransportes. Im Brennpunkt stehen Chlorid- und Kaliumkanäle sowie Kalium-Chlorid-Cotransporter. Mausmodelle ermöglichen entscheidende Einsichten in die Rolle intrazellulärer Chloridkanäle und ihrer Rolle bei der Endozytose und in die Rolle des KCl-Cotransports bei synaptischer Inhibition, Neurodegeneration und Taubheit. Die Gruppe konnte mehrere Erbkrankheiten molekular aufklären und pathophysiologisch erklären.

Die Forschung des Instituts für Neurale Signalverarbeitung (Pongs) befasst sich mit der strukturellen und funktionellen Charakterisierung von Ionenkanälen. Im Mittelpunkt stehen die Charakterisierung neuer Ionenkanalgene, die mit Herzrhythmusstörungen assoziiert sind, die Entwicklung geeigneter Tiermodelle zur Pathophysiologie von Kaliumkanal-Dysfunktionen und Struktur-Funktions-Untersuchungen zur Pharmakologie und zum Schaltverhalten von spannungsabhängigen Kaliumkanälen.

Forschungsschwerpunkt des Institutes für Zellbiochemie und Klinische Neurobiologie (Richter) ist die Reaktion von Neuronen auf extrazelluläre und intrazelluläre Signale. Extrazelluläre Signale werden u.a. an Neuropeptidhormon- und Geschmacksrezeptoren erforscht. Im Vordergrund stehen Untersuchungen der Expressionsmuster, die Identifizierung von Ligandenbindungsstellen sowie eine Charakterisierung der strukturellen Voraussetzungen einer internen Signalleitung in Säugerzellen. Als ein Beispiel für intrazelluläre Signalkaskaden werden die molekularen Mechanismen eines selektiven zytoplasmatischen mRNA-Transports in Dendriten und Axone analysiert. Eine dezentrale Proteinsynthese scheint zu einer individuellen Proteinausstattung einzelner zellulärer Subregionen und somit zur Entstehung und Veränderung morphologischer Muster und Polaritäten beizutragen. Gegenwärtig werden *cis*- und *trans*-agierende Faktoren des subzellulären mRNA-Transportprozesses in Neuronen untersucht.

Die Arbeiten des Instituts für Biosynthese Neuraler Strukturen (Schachner) beschäftigen sich mit der Aufklärung der Funktionen von Zellerkennungsmolekülen bei der Entwicklung des Nervensystems und bei der Regeneration nach Läsion. Sie befassen sich ferner mit der Induktion und Aufrechterhaltung von synaptischer Plastizität im erwachsenen Nervensystem. Genetisch veränderte Mäuse ermöglichen Erkenntnisse über menschliche Krankheiten. Neurale und embryonale Stammzellen und die Funktionen von Kohlehydraten bei der Feinregulation der Zellerkennung sind für diese Untersuchungen von besonderem Interesse.

Im Institut für Entwicklungsneurobiologie (Schaller) wird die Wirkungsweise des Neuropeptids Kopfaktivator (KA), seine Interaktion mit Rezeptoren und die Signaltransduktion erforscht. Im Säugetier wirkt KA neuroprotektiv und stimuliert die Zellteilung von neuroendokrinen Zellen und von neuronalen Vorläuferzellen. Auf der Suche nach dem hoch affinen G-Protein-gekoppelten KA-Rezeptor wird in heterologen Expressionssystemen eine Serie neuer Waisenrezeptoren auf Bindung und Wirkung von KA und anderer Liganden untersucht.

Die Forschergruppe Bach bearbeitet Fragestellungen der molekularen Mechanismen neuronaler Zellspezifizierung während der Embryogenese. Die Forschungen konzentrieren sich auf die Regulation einer Klasse von Transkriptionsfaktoren, die LIM Homeoproteine genannt werden, und die grundlegende Funktionen in der Entstehung und Differenzierung von Neuronen übernehmen. Ingolf Bach und Mitarbeiter konnten zeigen, dass die biologische Aktivität von LIM Homeoproteinen entscheidend durch Protein-Protein Wechselwirkungen mit verschiedenen Kofaktoren beeinflusst wird.

Die Forschergruppe Hoppe befasst sich mit der Identifizierung und Charakterisierung grundlegend neuer Komponenten des Ubiquitin/Proteasom-Systems, das in allen Eukaryonten den selektiven Proteinabbau vermittelt. Ein besonderer Schwerpunkt liegt dabei auf einer neuen Gruppe von Multiubiquitinierungsfaktoren oder E4 Enzymen. Interessanterweise ist ein Mitglied dieser Familie in der Entstehung einer frühen Form der Parkinson'schen Erkrankung (AR-JP) involviert. Darüber hinaus versucht die Arbeitsgruppe weitere Faktoren zu identifizieren, die spezifisch am neuronalen Proteinabbau in *C. elegans* und möglicherweise beim Menschen an der Entwicklung neurodegenerativer Erkrankungen beteiligt sind.

Die Forschergruppe Kneussel bearbeitet Fragestellungen der Sortierung und des Transports von Neurotransmitter-Rezeptoren in neuronalen Dendriten. Die Zielsteuerung dieser Proteine zu und von der Synapse sowie deren Verankerung an der postsynaptischen Spezialisierung sind

ein Schwerpunkt der Arbeiten. Zur Identifizierung von neuronalen Transportkomplexen werden biochemische Screening-Verfahren angewandt. Als Modellsystem für funktionelle Studien dienen die neuronale Zellkultur mit hippokampalen Neuronen und genetisch veränderte Mäuse.

Die Erregungsübertragung an zentralnervösen Synapsen wird häufig über spezielle G-Protein gekoppelte Rezeptoren reguliert. Im Fokus der Arbeiten der Forschergruppe Kornau stehen direkte Interaktionen von diesen metabotropen Rezeptoren, insbesondere dem $GABA_B$-Rezeptor, mit löslichen und membranständigen synaptischen Proteinen sowie ihre funktionellen Konsequenzen.

Die Forschergruppe Riethmacher beschäftigt sich vor allem mit Fragestellungen der Entwicklungsbiologie. Ein besonderer Schwerpunkt ist die Aufklärung der verschiedenen Funktionen des Neuregulin-Signal-Systems sowie des Transkriptionsfaktors Sox10 im peripheren Nervensystems mittels Mausmodellen. Außerdem wird eine Mauslinie zur in-vivo Zellablation mittels aktivierbarer Expression des Diphtherietoxins etabliert sowie die Funktionen der Caspase 8 im zentralen Nervensystem mittels des Cre-Lox P-Systems analysiert.

Die Forschergruppe Sander untersuchte die molekularen Mechanismen, mit deren Hilfe die Transkriptionsfaktoren die Entwicklung des zentralen Nervensystems und des Pankreas kontrollieren. Im Speziellen versuchte die Gruppe zu verstehen, wie Transkriptionsfaktoren die Entwicklung verschiedener Zelltypen vermitteln und welche Zielgene durch diese Faktoren aktiviert oder reprimiert werden. Der Gruppe gelang es, wichtige Transkriptionsfaktoren für die Entwicklung von Motorneuronen und von Insulin-produzierenden Zellen im Pankreas zu identifizieren. In der Berichtsperiode erhielt Maike Sander einen Ruf als Assistant Professor an die Universität von Kalifornien, Irvine.

Im Mittelpunkt der Forschung der Nachwuchsgruppe Schimmang steht die Entwicklung und Differenzierung des Gehörorgans. Dabei werden besonders die Rollen von Wachstumsfaktoren bei der Induktion des Innenohrs, beim Überleben von Haarsinneszellen und Gehörneuronen und beim Schutz vor Gehörschäden untersucht. Neben der Analyse von transgenen Mausmodellen steht der therapeutische Gentransfer von Wachstumsfaktoren in das Innenohr im Vordergrund.

Ziel- und Leistungsvereinbarung

Im Jahr 2000 konnte das ZMNH eine Ziel- und Leistungsvereinbarung mit der Behörde für Wissenschaft und Forschung, der Universität Hamburg und dem Fachbereich Medizin für einen Zeitraum von fünf Jahren abschließen. Dieser Abschluss hat dem ZMNH stabile Rahmenbedingungen und eine große Planungssicherheit gegeben, ohne die das ZMNH in Zeiten zunehmender Mittelknappheit sicherlich nicht hätte so erfolgreich arbeiten können. Entsprechend dieser Vereinbarung finanziert die Stadt Hamburg in Umsetzung des zwischen Bund und Land vereinbarten Zentrumskonzepts auch vier budget-finanzierte Forschergruppen, deren Leitungen jeweils international ausgeschrieben wurden bzw. werden.

2002 wurde das Universitätskrankenhaus verselbstständigt und erhielt eine neue Struktur. Im Rahmen der mit der Verselbstständigung verbundenen Strukturänderungen wurden die im Fachbereich Medizin angesiedelten Institute in Zentren zusammengefasst. Für das ZMNH bedeutet dies, dass das Institut des Gründungsdirektors des ZMNH (Prof.

Dr. D. Richter) nicht länger mit dem ZMNH assoziiert ist, sondern fünftes Institut des ZMNH geworden ist. Die mit der Erweiterung des ZMNH verbundenen Struktur-, Satzungs- und Raumfragen bedürfen allerdings noch einer endgültigen Klärung, insbesondere im Hinblick auf die vorgesehene Neubesetzung des Instituts Richter. Der Fachbereichsrat Medizin hat hierzu beschlossen, das Institut umzuwidmen in ein Institut für Neuroimmunologie und klinische Multiple-Sklerose-Forschung. Hiermit folgt das ZMNH auch einer Empfehlung seines wissenschaftlichen Beirats, ausgezeichnete Grundlagenforschung mit kliniknahen, krankheitsrelevanten Aspekten am Universitätsklinikum Eppendorf zu verbinden und maßgeblich zu fördern.

Die Verselbstständigung des UKE hat bedauerlicherweise nicht gleichzeitig auch die Ausweitung und Festschreibung der Selbstständigkeit des ZMNH vorangetrieben, so dass diese Zielsetzung der Vereinbarung, die eine wichtige Randbedingung für die bei hoch kompetitiver Forschung benötigte Effizienz und Flexibilität darstellt, noch nicht umgesetzt worden ist.

Ausblick

Das ZMNH konnte sich in der Vergangenheit als eines der auf seinen Arbeitsgebieten besten Forschungszentren etablieren. Neben der Kreativität und dem Engagement der ZMNH-Mitarbeiter beruht dieser Erfolg auch auf der guten Finanzierung durch die Stadt Hamburg und auf der Struktur des ZMNH – einer gelungenen Kombination größerer Institute, unabhängiger Nachwuchsgruppen, wissenschaftlicher Service-Einrichtungen und einer weitgehend selbstständigen Verwaltung. Nach nunmehr 16 Jahren seiner Geschichte ist das ZMNH an einer wichtigen Wegscheide angelangt. Chica Schaller und Dietmar Richter emeritieren. Wir hoffen, dass die Lücke, die sie hinterlassen, durch hervorragende Neubesetzungen rasch gefüllt werden kann. Ferner gilt es, in diesem Jahr eine neue Ziel- und Leistungsvereinbarung auszuhandeln, die entscheidend die weitere Entwicklung des ZMNH beeinflussen wird. Wir sind fest davon überzeugt, dass das exzellente Gründungskonzept des ZMNH mehr denn je aktuell und hervorragend geeignet ist, sich den neuen Herausforderungen der Hochschulmedizin zu stellen. Wir gehen davon aus, dass wir wiederum eine mehrjährige Ziel- und Leistungsvereinbarung abschließen können, um Planungssicherheit, Haushaltsstabilität und Stärkung der Selbstständigkeit zu gewährleisten und die herausragende Stellung des ZMNH als Forschungszentrum der Universität Hamburg am Fachbereich Medizin weiter zu festigen.

Herr Jentsch hat das ZMNH in den Jahren 2000 bis 2003 als Direktor geleitet. Ich möchte ihm für dieses Engagement herzlich danken.

April 2004, Olaf Pongs

Preface

The Center for Molecular Neurobiology is a research center of the University of Hamburg and is part of the Faculty of Medicine. Its research concerns questions of molecular neurobiology and related areas. In many cases, research results are swiftly applied to problems in medicine and human genetics. This process is greatly facilitated by the generation and analysis of transgenic and “knock-out” mice. These mouse models have led to the identification of novel disease

genes and have elucidated the pathophysiological basis of human disorders. In addition to research, the ZMNH is involved in teaching. The center carries out a two-year graduate course in molecular biology and participates in an international graduate study programme.

The ZMNH comprises four institutes which are headed by full professors (Jentsch, Pongs, Schachner, Schaller), as well as several research groups that are led by young researchers. In addition, the institute of the founding director D. Richter belongs to the ZMNH. All institutes and research groups are supported by several scientific service units. Furthermore, the largely independent administration of the ZMNH is crucial for flexible and efficient administrative procedures. A scientific advisory board evaluates the performance of the ZMNH in regular intervals and provides valuable advice.

The independent junior research groups are essential to the concept of the center. The City-State of Hamburg finances four junior research groups since 2002 (Kneussel, Riethmacher, Sander/Hoppe, Schimmang). Two additional junior research groups are entirely funded by the Deutsche Forschungsgemeinschaft (DFG). Ingolf Bach is a recipient of a Heisenberg Fellowship, and Hans-Christian Kornau has joined us at the end of 2001 as the head of a junior research group of the Sonderforschungsbereich 444 (a Collaborative Research Center of the DFG). Thus, six independent junior research groups are presently working at the ZMNH. During the reporting period, Maike Sander has accepted an appointment as assistant professor in the Department of Developmental and Cellular Biology at the University of California, Irvine, and Dietmar Kuhl has taken up the chair for Molecular Neurobiology at the Faculty of Biology of the Free University of Berlin. The Junior Research Programme of the ZMNH continues to be an extraordinary successful concept to support the scientific and professional careers of young, independently working scientists. Accordingly, several institutes of the ZMNH (Pongs, Richter, Schaller) have decided to extent this concept and to support adjunct junior research groups by providing space and money in their institutes for such groups. Sixteen years after its foundation in 1988, the ZMNH is now firmly integrated into the Hamburg research community. There is an ever increasing number of local scientific collaborations, in particular with groups of the University Clinics Eppendorf (UKE). In this respect, the various mouse models generated by researchers of the ZMNH play a prominent role. The broad expertise which is available at the UKE for various organ systems, is often invaluable in analysing their diverse and sometimes unexpected phenotypes.

The ZMNH is firmly integrated into several research networks, in which the ZMNH is often the driving force. Thus, the SFB 444 (*Basis of neuronal communication and signal transduction*, speaker: Jentsch) is largely centred at the ZMNH, as is the DFG graduate programme (*Neural signal transduction and its pathology,* speaker: Schaller). Groups of the ZMNH participate in the SFB 470 (*Glycostructures in biological systems*, speaker: Thiem), as well as in the Research Group *RNA transport*, (speaker: Richter). Recently, the ZMNH was selected as one of the five German Centers (speaker: Jentsch) focusing on the pathology of neurological diseases within the National Genome Network financed by the Federal Ministry for Research and Education. Besides five groups of the ZMNH it comprises the Human Genetics Institute of the UKE.

Research at the ZMNH

The ZMNH is now widely recognised as one of Germany's premier research institutes in neurobiology. The center's

scientific success is above all reflected in its publications, which often report breakthroughs in the respective areas. Moreover, in the period covered by the present report, scientists of the ZMNH have again been awarded several prestigious prizes. The Berliner professorship of Yale University, the Adolf-Fick Price, the Homer W. Smith Award, and the Gottschalk Lecture to Thomas Jentsch, the Bickel Price ot the German Cardiological Society to Dirk Isbrandt, the Young Research Award of the European Society of Anaestesiologists to Patrick Friederich, and the Finkelstein Price of the Northern German Society for Pediatry to Axel Neu.

Research at the ZMNH primarily tackles problems of molecular neurobiology. It focuses on the structure, function, and (patho)physiological importance of ion channels, development and differentiation of the nervous system, cell adhesion molecules, and synaptic plasticity. In addition, researchers of the ZMNH investigate other topics of cell and developmental biology, and are concerned with a broad spectrum of pathophysiological conditions and inherited diseases. Thus, important progress has been made in understanding the development of *hydra*, the development of the pancreas, in unravelling mechanisms of cardiac arrhythmia, hypertension, infertility, kidney diseases, mental retardation, polyneuropathies, and regeneration of the nervous system. The ZMNH has proved to be very efficient in the generation and analysis of genetic mouse models, and several breakthroughs were achieved in the molecular genetics and pathophysiology of human inherited diseases.

The Institute for Molecular Neuropathology (headed by Thomas Jentsch) focuses on ion transport processes, in particular on their role in physiology and disease. Its research is primarily concerned with chloride and potassium channels, and, more recently, potassium chloride cotransport. A number of knock-out mouse models has provided crucial insights into the roles of intracellular chloride channels in vesicle trafficking and endocytosis, as well as into the role of KCl-cotransport in synaptic inhibition, neurodegeneration and deafness. The group identified several human disease genes and clarified the pathophysiology of these disorders.

The research at the Institute for Neural Signal Transduction (Olaf Pongs) is focused on structural and functional studies of ion channels, in particular potassium channels. The institute is involved in human genetic screens for ion channel genes associated with various heart diseases, in the development of animal models to study potassium channel dysfunctions related to vasoregulation, learning and epilepsy, and in structure-based studies of potassium channel function.

Research in the Institute of Cell Biochemistry and Clinical Neurobiology (Dietmar Richter) is focussed on studying responses of neurons to extra and intracellular signalling. In particular, effects of extracellular signals on neuropeptide-hormone and, respectively, taste receptors are investigated. Of special interest are analyses of expression profiles, identification of ligand-binding sites, as well as of structural domains linking the extracellular signal to intracellular signalling cascades in eukaryotic cells. As an example for an intracellular signalling cascade, the molecular mechanisms of selective cytoplasmic mRNA transport to dendrites and axons are analyzed. Apparently, a decentralized protein biosynthesis contributes to the distinct protein composition in subcellular regions associated with genesis and plasticity of morphological patterns and cell polarity. Presently, both cis- and trans-acting factors are investigated that influence specifically subcellular mRNA transport in neurons.

Research in the Institute for Biosynthesis of Neural Structures (Melitta Schachner) focuses on the function of neural recognition molecules during development of the nervous system, and during regeneration after a lesion and induction and maintenance of synaptic plasticity in the adult. Genetically modified mice as models for human diseases and neural and embryonic stem cells and functions of carbohydrates in the fine regulation of cell recognition are of particular interest to these investigations.

One of the external signals that influences early events in neuronal and neuroendocrine development, is the neuropeptide head activator (HA). In the report period the research of the Institute for Developmental Neurobiology (headed by Chica Schaller) concentrated on characterising new members of the VPS-10 domain-containing and G-protein coupled receptor (GPR) families as candidates for HA signal transduction. Their interaction with HA and other ligands was studied by using various heterologous expression systems.

The Bach research group investigates questions concerning molecular mechanisms underlying neuronal cell fate specification during embryogenesis. The research interests focus on the regulation of LIM homeodomain proteins, a class of transcription factors that is crucial for the development of neuronal structures and cell types. This group has demonstrated that the biological activity of LIM homeodomain proteins is critically regulated by protein-protein interactions with several associated cofactors.

The Hoppe research group studies components of the ubiquitine/proteasome system, which is a key player in regulated protein degradation in all eukaryotic cells. The group focuses on new ubiquitination factors (E4 enzymes). Of special interest is that members of this protein family may be involved in the development of an early onset form of Parkinson's disease. Furthermore, the research group of Thorsten Hoppe tries to identify additional proteins, which are specifically involved in degradation of neuronal proteins in *C. elegans* and, possibly, may be linked to neurodegenerative diseases in humans.

The Kneussel research group studies mechanisms of dendritic sorting and dendritic transport of neurotransmitter receptors. The focus lies on the transport of these proteins to the synapse and their association with postsynaptic specialisations. As model system for these studies serve cultured hippocampal neurons as well as genetically altered mice, in which the group attempts to identify neuronal transport complexes, in particular by using proteomics derived biochemical screening techniques.

Synaptic transmission in the central nervous system is frequently regulated by specific G-protein-coupled receptors. The studies of the Kornau research group are focused on direct interactions of these metabotropic receptors, in particular the $GABA_B$ receptor, with both soluble and membrane-bound synaptic proteins as well as on their functional consequences.

The research group of Dieter Riethmacher is engaged in analysing questions of developmental biology. Using mouse models the group focuses on unravelling the functions of the neuregulin-signalling-system and the sox10 transcription factor in PNS development. Additionally the Cre-lox-P-system is used to analyse functions of the caspase 8 in CNS development and pathology and a mousestrain that enables specific cell ablation through an inducible diphtheria-toxin A fragment is established.

The Sander laboratory aimed to understand the molecular mechanisms by which several classed of transcription factors

co-ordinate development of the mammalian central nervous system and pancreas. In their research they combined genetic, molecular, and genomic approaches to gain insight into the molecular mechanisms of synergistic activation and repression of gene expression. In this, the research group identified key transcriptional regulators of spinal cord motor neuron development, as well as of insulin-producing cells in the pancreas. During the reporting period, the group moved to the Department of Developmental and Cellular Biology at the University of California, Irvine.

The central interest of the research group headed by Thomas Schimmang is the formation and differentiation of the auditory organ. The roles of growth factors during the induction of the inner ear, the survival of sensory hair cells and the auditory neurons and their protection from ototoxic damage are studied. Next to the analysis of transgenic mouse models, his group is interested in therapeutic gene transfer of growth factors into the inner ear.

A stable framework for the further development of the Center

In 2000, the ZMNH negotiated an important agreement with the Ministry of Science and Research of the City-State of Hamburg, the president of the University, and the dean of the Faculty of Medicine. The five years duration of this agreement provided, in times of decreasing resources, stable basic funding by both the City-State of Hamburg and the UKE. Accordingly, the City-State of Hamburg finances now four junior research groups at our center. Without the stable financial framework provided by the City-State of Hamburg the Center would not have been able to be as successful as it has been in the recent years.

In 2002, the City-State of Hamburg initiated an important structural change by transforming the University Clinics (and with it the Faculty of Medicine) into an independently operating entity. As a consequence, the Faculty of Medicine reorganized itself into centers, and the institute of our founding director D. Richter was joined to our center as fifth institute. This expansion of the ZMNH has not yet been formally completed. This fall, D. Richter will retire. The Faculty of Medicine decided to find a successor for his institute in the field of neuroimmunology and clinical multiple-sclerosis research. This may open up the possibility for the ZMNH to co-operate in the future even stronger with clinically oriented institutes at the UKE in elucidating and tackling the bases of human neurological diseases in accordance with a recommendation of the scientific advisory board of the ZMNH.

The structural changes in the organization of the UKE did unfortunately not extend to the much desired gain of more administrative independence of the ZMNH as it was envisaged since its founding days, and which has been a fundamental concept for its attractiveness, competitiveness, and scientific success.

Outlook

Within the past 16 years, the ZMNH established itself as one of the leading research centers in its field. This remarkable success is not only due to the creativity and hard work of its scientists, but also to the rather generous basic financing by the City-State of Hamburg and to the structure of the ZMNH-which judiciously combines rather large institutes with independent young research groups, service groups, and a largely autonomous administration. The ZMNH has to renegotiate the above-mentioned agreement within the newly established structure of the UKE. We hope that

the larger autonomy of the ZMNH agreed upon in 2000 will be implemented with this new agreement, which may be a good opportunity to further strengthen the founding concept of the ZMNH and to meet future challenges in a rapidly changing University. Given the achievements of the Center and its excellent facilities, I am optimistic that the ZMNH will continue to provide outstanding research and teaching facilities in molecular neurobiology. Soon, Chica Schaller and Dietmar Richter will retire as Directors of their respective institutes. To fill the substantial gaps that their retirements will create, will be an additional challenge for the future of the ZMNH.

Thomas Jentsch served as Director of the ZMNH from 2000 to 2003. I would like to take this opportunity to thank him very much for his efforts and input during that time.

April 2004, Olaf Pongs

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Research Projects

Institut für Biosynthese neuraler Strukturen

Melitta Schachner Camartin

Formation of the appropriate connections among nerve cells is essential for the correct and efficient functioning of the nervous system. It is through very specialized interactions between the different neural cell types that such connections are formed during development, maintained or modified in the adult, and reformed or even prevented after trauma.

Cell surface and extracellular matrix molecules that have been recognized to mediate such interactions are now being implicated in such diverse phenomena as neural induction, neural cell proliferation, neuronal migration, neurite outgrowth, synaptogenesis, signal transduction between neurons and glia, and finally, the capacity of neurons to regenerate or not. For instance, how does a neuron sense where to position its cell body, into which direction to send out its neurites, and when to engage in stable connections or to destabilize such connections under conditions requiring plasticity, such as learning and memory.

Thus, not only recognition between interacting cells is called for, but mechanisms must be implemented that transduce cell surface triggers - resulting from recognition - into sensible and sensitive intracellular responses that guide a cell's ultimate behaviour in the intricate context of network activities. The aim of our research is to understand the molecular events that mediate communication among cells in the nervous system not only during the ontogenetic formation of connections, but also in the adult nervous system under conditions of synaptic plasticity and trauma.

1. The L1 family of neural cell adhesion molecules

Ivayla Apostolova, Stephan Kaizik, Ralf Kleene, Nicole Meyer, Mounir Mzoughi, Nadine Salis, Ann-Britt Steen, Meike Zerwas

The neural cell adhesion molecule L1 is a multifunctional molecule that has been implicated in neuronal migration, neurite extension and fasciculation, myelination in the peripheral nervous system, and synaptic plasticity. It is the founding member of a family comprising several L1-like molecules, all of which enhance neurite outgrowth.

The L1-like molecules are present in overlapping and distinct subpopulations of neurons at different stages of development and may be important determinators of specific axon outgrowth patterns during development. Structure-function relationships of the different domains of L1 have been characterized and the molecular associations of L1 with other neural recognition molecules, including NCAM, CD24, and laminin have been investigated.

L1 and the close homolog of L1 (CHL1) are proteolytically processed by different metalloproteases that subserve different functions in second messenger mediated neuronal survival and neurite outgrowth. It is now well documented that L1 induces neurite outgrowth and neuronal survival in vitro. Since L1, a prominent glia-associated neurite outgrowth promoting molecule in the peripheral nervous system, is absent in the central nervous system on glial cells, we evaluated its neurite outgrowth promoting role in the central nervous system using transgenic mice that overexpress L1 in glial cells during crucial stages of regeneration after a

lesion. In this transgenic mouse, neuronal, in particular dopaminergic differentiation and survival and the learning performance in the Morris water maze test are enhanced.

Analysis of an L1-deficient mouse mutant generated by homologous recombination has revealed this mutant to be a very good animal model for the inherited human diseases caused by mutations in the L1 gene that are now summarized under the name of CRASH syndrome (comprising mental retardation, aphasia, shuffling gate, adducted thumbs, spastic paraplegia type I, and hydrocephalus). A knock-out mouse mutant deficient in the close homologue of L1 (CHL1) shows a much less severe phenotype. Conditional knockout mutants have been generated and double knock-out mutants carrying defects in genes of the L1 family are being analyzed to further probe the functions of these molecules.

2. Neural recognition molecules and signal transduction

Anja Behrendt, Claas Cassens, Athena Chalaris, Claudia Friedrich, Ina Kalus, Gabriele Loers, Ingo Meier, Daniel Novak, Ambrish Saxena, Daniela Schneeberger, Tanja Schneegans, Thomas Tilling, Ann-Kathrin Tranziska, Gerrit Wolters

The identification and characterization of intracellular signaling cascades activated by homophilic (self binding) or heterophilic (binding to other molecules) interactions of cell-adhesion molecules such as L1 or the neural cell adhesion molecule NCAM are of central importance for the understanding of adhesion molecule-mediated neurito-genesis and growth cone repulsion.

One of the advances in our group in the recent years was to identify the essential role of lipid rafts for NCAM-mediated signaling processes. The transmembranous isoforms of NCAM can be palmitoylated on intracellular cysteines and are thereby directed into these lipid rafts. The physiological consequences of this lipid raft association turned out to be very important. First, we demonstrated that NCAM-dependent neurite outgrowth is mediated via an „outside-in“ co-signaling mechanism involving the Fyn-FAK pathway and the FGF receptor. While activation of the Fyn-FAK pathway critically depends on the association of NCAM with lipid rafts, probably due to the co-localization of NCAM and the Fyn kinase in lipid rafts, the NCAM-dependent activation of the FGF receptor is mediated in the non-raft compartment. Secondly, we showed that surface localization of G protein inwardly rectifying K^+ channels (Kir3 channels) is controlled by palmitoylated NCAM isoforms in the trans-Golgi network, a process that can be called an „inside-out“ signaling of NCAM. These results could explain how cell adhesion molecules are involved in the regulation of neural activity.

3. Prion protein and amyloid precursor protein as recognition molecules

Vasudharani Devanathan, Isabel Köhlitz, Friedhelm Maywald, Antonella Santuccione, Carsten Schmidt, Helen Strekalova

The cellular form of prion protein (PrPc) is a glycosylphosphatidylinositol (GPI) anchored ubiquitous cell surface glycoprotein. Conversion of PrPc to an abnormal conformer (PrPSc) is a central event in the pathogenesis of prion diseases, such as Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattles and

scrapie in sheep. Several recently reported observations are consistent with a function of PrPc as a cell adhesion molecule. We confirmed this notion showing that the recognition molecule-related HNK-1 carbohydrate is linked to the protein backbone of PrPc via N-linked glycans. Additionally, we showed that PrPc induces neurite outgrowth and neuronal survival in cell culture of primary neurons via a yet unknown heterophilic receptor involving different signal transduction pathways. Our current studies are focused on studying this aspect that it is essential for PrPc mediated neurite outgrowth, neuronal survival and synaptic plasticity, and the associated triggering of signal transduction mechanisms.

The amyloid precursor protein, leading to Alzheimer's disease when not correctly cleaved, is studied in a similar manner.

4. Carbohydrates and the fine tuning of cell interactions

Nuray Akyüz, Frauke Brendel, Tatjana Makhina, Sandra Nickel, Olga Simova

We are engaged in studies on different glycans that are carried by partially overlapping sets of glycoproteins, many of which have been shown to be neural recognition molecules. Some of these neural recognition molecules, e.g. L1, MAG, NCAM and basigin, are capable to bind distinct carbohydrate structures, thus functioning as lectins (Figure 1). In vitro assays have shown that glycans themselves are involved in different aspects of cell adhesion, cell migration, outgrowth of neuritic and astrocytic processes as well as in synapse formation and synaptic plasticity. We focus our

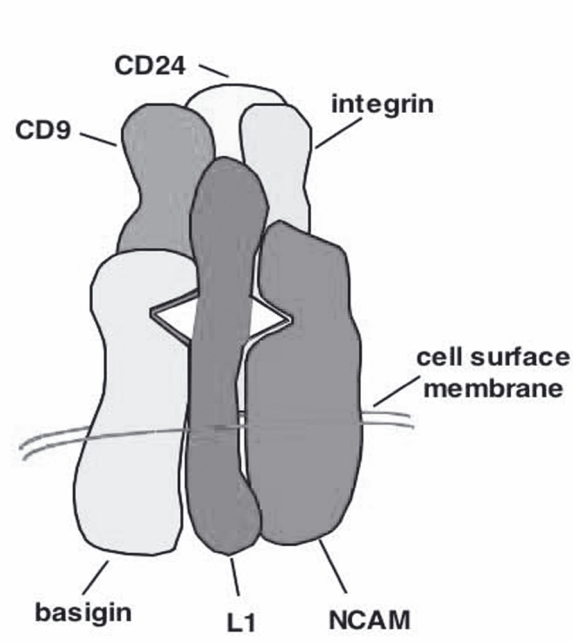

Figure 1: Cell surface recognition molecules bind to each other to form complexes that function as recognition and signaling platforms. L1 docks into NCAM or basigin via oligomannosides. L1 is not only a donor of glycans but also an acceptor for 2,3-neuraminic acid from the mucin-like recognition molecule CD24.

studies on several functionally important glycans, among them the HNK-1 carbohydrate, oligomannosidic carbohydrates, the unusual alpha-2,8-linked polysialic acid (PSA), the Lewis^x epitope and alpha-2,3-linked sialic acid on O-glycans. All of these carbohydrates are involved in the modulation and fine tuning of cell interactions. We are presently looking for receptors of these molecules by using immunological, biochemical and molecular biological

techniques. Furthermore, the regulatory mechanisms underlying the synthesis and degradation of these functionally important carbohydrates are investigated. We are also identifying carbohydrate peptide-mimetics with the view to use these as surrogate carbohydrates to trigger or block cell interactions: they are more easily obtained in large amounts than many structurally complex carbohydrates and can be manufactured as better binding ligands with higher metabolic stability.

The HNK-1 carbohydrate is a well-characterized example of a protein- and lipid-linked oligosaccharide. This epitope is regulated in its expression independently of the protein backbone, is phylogenetically conserved, and is functional in cell-cell and, particularly, cell-substrate interactions. Interestingly, the sulfate group is essential for most of the functions contributed by this epitope. The enzyme transferring the sulfate group to the oligosaccharide backbone, the HNK-1 sulfotransferase (HNK-1 ST), has previously been cloned and the HNK-1 ST knockout mutant has been generated and characterized. Based on the homology to the HNK-1 sulfotransferase we and others have identified and cloned six more members of this enzyme family. The sulfotransferases GalNAc-4ST1 and GalNAc-4ST2 have been shown to synthesize sulfated beta1-4-linked GalNAc found on the GGnM epitope characteristic of glycopeptide hormones of the pituitary and to add sulfate to non-terminal beta1-4-linked GalNAc found on chondroitin and dermatan. The sulfotransferases C4ST1, C4ST2, C4ST3 and D4ST1 confer sulfate to beta1-4-linked GalNAc on chondroitin and dermatan.

5. Immunoglobulin superfamily molecules and their roles in organization of the pre- and post-synaptic machinery

Aksana Andreyeva, Vsevolod Bodrikov, Yana Chernyshova, Nainesh Katagihallimath, Iryna Leshchyns'ka, Aparna Shetty, Vladimir Sytnyk, Avinash Thirumalai

It is well established that neural cell adhesion molecules accumulate at synapses and are implicated in certain forms of synaptic plasticity such as long term potentiation. Neural cell adhesion molecules are involved in cell contact stabilization due to homophilic and heterophilic binding to molecules on the adjacent cell surface membranes. Recently, we found that the life time of the contacts formed by hippocampal neurons derived from NCAM deficient mice is reduced when compared to wild type neurons providing direct evidence for the importance of NCAM in the stabilization of interneuronal contacts. Since cell adhesion molecules themselves are stabilized at synapses due to their homophilic or heterophilic interactions, they can provide cues for accumulation of certain synaptic proteins and even organelles at these sites. Recently, we confirmed this notion by showing that NCAM is involved in the positioning of trans-Golgi network (TGN) derived organelles in the vicinity of contact sites before transformation of these contacts into synapses (Figure 2).

Another function of cell adhesion molecules in synapses is the regulation of distinct signaling cascades resulting in activation of different kinases and leading to changes in gene expression. Recently, we showed that in response to activation, NCAM utilizes βI spectrin to translocate PKCα2

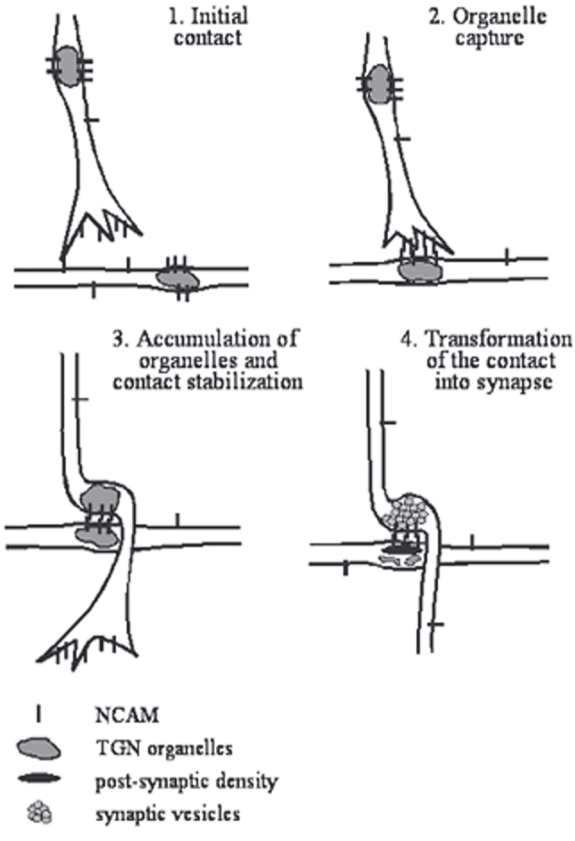

Figure 2: A model of NCAM mediated accumulation of TGN organelles at sites of initial cell contact followed by synaptic differentiation.

to lipid rafts where this kinase activates its substrates. Current work aims at further investigations on the role of NCAM, L1 and CHL1 in accumulation of synaptic proteins at synapses. Furthermore, we want to analyze how NCAM, L1 or CHL1 modulate the functions of their putative synaptic binding partners, thereby being involved in regulation of the pre- or post-synaptic signaling machinery.

6. Recognition molecules and synaptic plasticity

Olena Bukalo, Alexander Dityatev, Martin Hammond, Andrey Irintchev, Eka Lepsveridze, Olexandr Nikonenko, Dmytro Puchkov, Adriana Stan, Luminita Stoenica

A major interest is to understand how the strength of synaptic connections is modified by neuronal activity. At the molecular level, extracellular matrix molecules (tenascin-R and -C and chondroitin sulfate proteoglycans) and cell adhesion molecules of the immunoglobulin superfamily (NCAM, L1, CHL1) are the focus of our research. To evaluate the contributions of these molecules to different aspects of synaptic plasticity in juvenile and adult mice, we are using several approaches and experimental models.

We have analyzed several forms of hippocampal synaptic plasticity in mutants deficient in the above mentioned molecules. Our results show that L1 and CHL1 constitutive deficient mice have normal long-term potentiation (LTP) in the CA1 region of the hippocampus, whereas NCAM, tenascin-C and tenascin-R mutants show reduced LTP in CA1. Long-term depression (LTD) in CA1 is reduced in NCAM and tenascin-C mutants but appears to be normal in tenascin-R mutants. No abnormalities in LTP in the CA3 region of mutants deficient in tenascin-C and tenascin-R were found. Thus, our data demonstrate that both cell surface and extracellular matrix molecules are involved in different

forms of hippocampal synaptic plasticity. Analysis of the underlying mechanisms revealed that impairments in LTP and LTD in tenascin-C deficient mice are related to a deficit in L-type Ca^{2+} channel-dependent signaling, whereas tenascin-R deficient mutants show impaired perisomatic inhibition and a modified threshold for induction of LTP.

Since many biological functions of recognition molecules are mediated by carbohydrates associated with these molecules, we analyzed synaptic plasticity in mice deficient in polysialyltransferase ST8SiaIV (producing polysialic acid associated exclusively with NCAM) and HNK-1 sulfotransferase (adding the HNK-1 carbohydrate to several recognition molecules), and after injection of antibody to HNK-1 or enzymatic removal of chondroitin sulfates in slices of wild type mice. ST8SiaIV-deficient mutants show an age-dependent reduction in LTP and LTD in CA1, which correlates with an age-dependent decline in expression of polysialic acid in ST8SiaIV-/- mutants. HNK-1 sulfotransferase deficient mice show moderate reduction in LTP and an increase in basal excitatory synaptic transmission, a phenotype found in tenascin-R deficient mice. The HNK-1 carbohydrate carried by tenascin-R interacts with the $GABA_B$ receptor and reduces its activity. Injection of the HNK-1 antibody increased LTP in CA1, most likely via reduction of perisomatic inhibition in the CA1 region. Enzymatic removal of chondroitin sulfates impaired both LTP and LTD in CA1, implicating this important carbohydrate for the first time in the regulation of synaptic plasticity.

Impairment of perisomatic inhibition in tenascin-R deficient mice and by HNK-1 antibody stimulated our interest to study regulation of inhibitory synaptic transmission by other recognition molecules. Recently, we found that in L1-deficient mice perisomatic inhibitory currents and the density of symmetric inhibitory synapses in the CA1 region of the hippocampus are reduced as in tenascin-R deficient mutants. Since tenascin-R and -C and chondroitin sulfate proteoglycans accumulate in the extracellular matrix surrounding some inhibitory interneurons in so-called perineuronal nets, we will attempt to unravel how these molecules affect different aspects of interneuronal activity and, thus, possibly shed light on the functions of perineuronal nets.

7. Neural recognition molecules and behavior

Alexander Angerer, Jörg Brandewiede, Fabio Morellini, Elena Oulianova, Anja Schrewe, Oleg Senkov

Bridging the gap between molecules and behavior is one of the most interesting and, at the same time, challenging tasks in neuroscience. The correlation between molecular mechanism and functional output at the behavioral level in vivo is complicated by the fact that behavior is the output of the complex interaction of many systems (nervous-, sensory-, endocrine-, immune-, muscular systems), regulated by the constant cross-talk between gene and environment throughout ontogenic development. The role of several neural cell adhesion molecules is studied mainly through two approaches: 1) Behavioral analysis of transgenic mice knocked out constitutively or conditionally for or overexpressing the gene of a certain neural cell adhesion molecule; 2) Analysis of differential cell activity specifically induced by behavioral events known to lead to neuronal plasticity, such as learning and novelty.

After preliminary observations on general health, home cage behavior, sensory abilities and motor functions, more specific behavioral tasks are conducted to test specific hypotheses on behavioral parameters of transgenic mice: a battery of tests is run to understand whether intra-sexual competition, exploratory behavior, reactivity to novelty (novelty seeking behavior) and anxiety may be altered due to genetic manipulation. Alteration of cognitive functions is tested in several learning paradigms such as the passive or active avoidance, fear conditioning, water maze, win-shift, Barnes maze and spatial object recognition tasks. We are currently analyzing mice constitutively and conditionally deficient for the neuronal cell adhesion molecules NCAM and L1 and for the extracellular matrix proteins tenascin-R and tenascin-C.

It has been proposed that “immediate early” genes coding for transcription-regulating factors may play a significant role in long term consolidation of plastic changes in neurons. By creating two groups of C57BL/6J mice in consideration of their performance in a variant of the Barnes maze (“fast learners” and “slow learners”), we are analyzing whether the expression of the immediate early genes c-fos and arg (by in-situ hybridization analysis) immediately after a new learning experience might differ due to the learning abilities at the individual level. The behavioral experiments are designed such to dissect learning-induced from novelty-induced gene expression. Possible difference in c-fos and arg expression after a learning event as well as after an anxiogenic event will also be analyzed in NCAM and L1 constitutively and conditionally deficient mice.

8. Stem cells and neural transplantation

Christian Bernreuther, Jian Chen, Marcel Dihné, Soo Yuen Leong, Mirjam Sibbe, Yuliya Tereshchenko

The long-term aim of these studies is to evaluate the use of such cells manipulated to express recognition molecules for the treatment of non-inflammatory dysmyelinating or demyelinating diseases. To test the capacity of neural stem cells to differentiate into myelinating oligodendrocytes, we have isolated such cells from striata or spinal cords of transgenic mouse embryos ubiquitously expressing enhanced green fluorescent protein (EGFP). The capacity of neural stem cells to differentiate into myelin-forming oligodendrocytes in vivo was tested by transplanting them into (i) the retina of wild-type mice containing the unmyelinated proximal segments of retinal ganglion cell axons and (ii) the lateral ventricle of severely hypomyelinated mutant mice deficient in the myelin-associated glycoprotein (MAG) and the non-receptor-type tyrosine kinase Fyn. In both experimental models, transplanted progenitor cells showed widespread integration into the host tissue. Cells transplanted into the retina of wild-type mice or the ventricle of MAG/Fyn double mutants showed preferential integration into the retinal nerve fiber layer or diverse white matter tracts, respectively.

Donor-derived cells differentiated into a variety of morphologically distinct cell types. A significant fraction of these cells was identified as oligodendrocytes, which myelinated unmyelinated host axons in both transplantation paradigms. Differentiated oligodendrocytes and myelin were still detectable in the host tissue six months after

transplantation, the latest time point investigated. Remarkably, prolonged survival periods of experimental animals resulted in a significant increase in the number of donor-derived oligodendrocytes and the area being myelinated. Recently, we found that the neural cell recognition molecule L1 influences CNS precursor cell proliferation and differentiation. We also investigate the effects of other recognition molecules, such as tenascin-C, tenascin-R and CHL1 on precursor cell behaviour. We use organotypic hippocampal cultures to study precursor cell behaviour in a more complex system. In vivo studies have examined CNS precursor cells expressing recognition molecules on morphological and functional regeneration in animal models of spinal cord injury, Huntington´s chorea, stroke and Parkinson´s disease.

9. Recognition molecules and axon growth in the nervous system of zebrafish

Catherina Becker, Thomas Becker, Anselm Ebert, Julia Feldner, Jörn Schweitzer

Zebrafish offer the unique opportunity to analyze axon growth both during early vertebrate development and during axon regeneration in the adult central nervous system. Embryos are transparent and their relatively simple scaffold of primary axons has been described in detail. The availability of expressed sequence tags and sequencing of the zebrafish genome, which is projected to be complete in the near future, provides easy access to genes of interest, and new methods to perturb gene function are being devised for this important model organism. Our focus is on cell recognition molecules that are important for axonal growth both on the axonal cell surface (L1.1, L1.2, NCAM, and other members of the immunoglobulin superfamily) and in the extracellular matrix (tenascins, proteoglycans). Functionally important unusual glycans attached to these molecules, such as the HNK-1 carbohydrate (Figure 3), oligomannosides, and polysialic acid are also investigated with regard to their possible role as fine tuners of cell interactions. We perturb expression of recognition molecules in vivo by microinjecting RNA (overexpression and knock-down), specific enzymes, peptides, and antibodies into fertilized eggs or embryos. Subsequently, we analyze aberrations of axon growth in these embryos using time-lapse video-microscopy in transgenic fish and immunohistochemical labeling of specific axons.

The recognition molecules under study are not only investigated during development, but also in regeneration and synaptic plasticity in the adult, when some of the ontogenetic mechanisms are recapitulated, at least to some extent. In contrast to mammals, which are unable to regenerate injured axons in their central nervous system, adult zebrafish show an impressive regenerative capacity of their central axons. We therefore study recognition molecules, which have functions for developmental axon growth also in axon regeneration. The two systems we are working on are regeneration of optic axons and of supraspinal descending axons in adult zebrafish. Regeneration of these fibers is analyzed in detail by axonal tracing. The distribution and regulation of recognition molecules is visualized using immunohistochemistry and in situ hybridization. The interactions of regenerating axons with recognition molecules are studied in vivo and in organotypic cell culture. Using these approaches in this model vertebrate we hope to gain insights into important

developmental processes and at the same time into the determinants of repair in the central nervous system after injury.

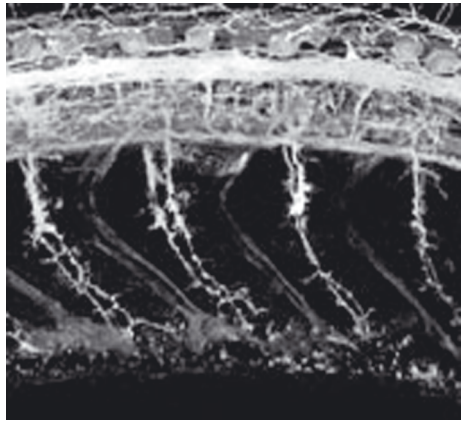

Figure 3: Confocal image stack of the nervous system in the trunk region of a zebrafish larva 24 hours after fertilization labeled with antibody to the HNK-1 carbohydrate. Two ventrally directed axons of the caudal primary motor neurons per trunk segment are prominently labeled. Rostral is left, dorsal is up.

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Publications

(1) Geyer, H., Bahr, U., Liedtke, S., Schachner, M. and Geyer, R. (2001). Core structures of polysialylated glycans present in neural cell adhesion molecule from newborn mouse brain. Eur. J. Biochem. 268, 6587-6599.

(2) Rollenhagen, A., Czaniera, R., Albert, M., Wintergerst, E.S. and Schachner, M. (2001). Immunocytological localization of the HNK-1 carbohydrate in murine cerebellum, hippocampus and spinal cord using monoclonal antibodies with different epitope specificities. J. Neurocytol. 30, 337-351.

(3) Sytnyk, V.N., Dityatev, A.E. and Korogod, S.M. (2001). Distribution of cell adhesion molecules (CAMs) along the branching neurite surface: model inherited effects of the branch diameter and mode of CAM transport. Neurophysiology 33, 15-19.

(4) Becker, C.G. and Becker, T. (2002). Repellent guidance of regenerating optic axons by chondroitin sulfate glycosaminoglycans in zebrafish. J. Neurosci. 22, 842-53.

(5) Becker, T., Ostendorff, H.P., Bossenz, M., Schluter, A., Becker, C.G., Peirano, R.I. and Bach, I. (2002). Multiple functions of LIM domain-binding CLIM/NLI/Ldb cofactors during zebrafish development. Mech. Dev. 117, 75-85.

(6) Chou, D.K., Schachner, M. and Jungalwala, F.B. (2002). HNK-1 sulfotransferase null mice express glucuronyl glycoconjugates and show normal cerebellar granule neuron migration in vivo and in vitro. J. Neurochem. 82, 1239-1251.

(7) Cifuentes-Diaz, C., Faille, L., Goudou, D., Schachner, M., Rieger, F. and Angaut-Petit, D. (2002). Abnormal reinnervation of skeletal muscle in a tenascin-C-deficient mouse. J. Neurosci. Res. 67, 93-99.

(8) Debiec, H., Kutsche, M., Schachner, M. and Ronco, P. (2002). Abnormal renal phenotype in L1 knockout mice: a novel cause of CAKUT. Nephrol. Dial. Transplant. 17 Suppl 9, 42-44.

(9) Delling, M., Wischmeyer, E., Dityatev, A., Sytnyk, V., Veh, R.W., Karschin, A. and Schachner, M. (2002). The neural cell adhesion molecule regulates cell-surface delivery of G-protein-activated inwardly rectifying potassium channels via lipid rafts. J. Neurosci. 22, 7154-7164.

(10) von der Ohe, M., Wheeler, S.F., Wuhrer, M., Harvey, D.J., Liedtke, S., Muhlenhoff, M., Gerardy-Schahn, R., Geyer, H., Dwek, R.A., Geyer, R., Wing, D.R. and Schachner, M. (2002). Localization and characterization of polysialic acid-containing N-linked glycans from bovine NCAM. Glycobiology 12, 47-63.

(11) Dong, L., Chen, S., Richter, M. and Schachner M. (2002). Single-chain variable fragment antibodies against the neural adhesion molecule CHL1 (close homolog of L1) enhance neurite outgrowth. J. Neurosci. Res. 69, 437-447.

(12) Ekici, A.B., Oezbey, S., Fuchs, C., Nelis, E., Van Broeckhoven, C., Schachner, M. and Rautenstrauss, B. (2002). Tracing Myelin Protein Zero (MPZ, P0) in vivo by construction of P0-GFP fusion proteins. BMC Cell Biol. 3, 29.

(13) Evers, M.R., Salmen, B., Bukalo, O., Rollenhagen, A., Bosl, M.R., Morellini, F., Bartsch, U., Dityatev, A. and Schachner, M. (2002). Impairment of L-type Ca2+ channel-dependent forms of hippocampal synaptic plasticity in mice deficient in the extracellular matrix glycoprotein tenascin-C. J. Neurosci. 22, 7177-7194.

(14) Gimnopoulos, D., Becker, C.G., Ostendorff, H.P., Bach, I., Schachner, M. and Becker, T. (2002). Expression of the zebrafish cell recognition molecule F3/F11/contactin in a subset of differentiating neurons is regulated by cofactors associated with nuclear LIM domains. Mech. Dev. 119, 135-141.

(15) Kang, H.G., Evers, M.R., Xia, G., Baenziger, J.U. and Schachner, M. (2002). Molecular cloning and characterization of chondroitin-4-O-sulfotransferase-3: A novel member of the HNK-1 family of sulfotransferases. J. Biol. Chem. 277, 34766-34772.

(16) Macias, M., Fehr, S., Dwornik, A., Sulejczak, D., Wiater, M., Czarkowska-Bauch, J., Skup, M. and Schachner, M. (2002). Exercise increases mRNA levels for adhesion molecules N-CAM and L1 correlating with BDNF response. Neuroreport 13, 2527-2530.

(17) Michelson, P., Hartwig, C., Schachner, M., Gal, A., Veske, A. and Finckh, U. (2002). Missense mutations in

the extracellular domain of the human neural cell adhesion molecule L1 reduce neurite outgrowth of murine cerebellar neurons. Hum. Mutat. 20, 481-482.

(18) Montag-Sallaz, M., Schachner, M. and Montag, D. (2002). Misguided axonal projections, neural cell adhesion molecule 180 mRNA upregulation, and altered behavior in mice deficient for the close homolog of L1. Mol. Cell. Biol. 22, 7967-7981.

(19) Niethammer, P., Delling, M., Sytnyk, V., Dityatev, A., Fukami, K. and Schachner, M. (2002). Cosignaling of NCAM via lipid rafts and the FGF receptor is required for neuritogenesis. J. Cell Biol. 157, 521-532.

(20) Ourednik, J., Ourednik, V., Lynch, W.P., Schachner, M. and Snyder, E.Y. (2002). Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. Nat. Biotechnol. 20, 1103-1110.

(21) Pavlou, O., Theodorakis, K., Falk, J., Kutsche, M., Schachner, M., Faivre-Sarrailh, C. and Karagogeos, D. (2002). Analysis of interactions of the adhesion molecule TAG-1 and its domains with other immunoglobulin superfamily members. Mol. Cell. Neurosci. 20, 367-381.

(22) Pujol, A., Hindelang, C., Callizot, N., Bartsch, U., Schachner, M. and Mandel, J.L. (2002). Late onset neurological phenotype of the X-ALD gene inactivation in mice: a mouse model for adrenomyeloneuropathy. Hum. Mol. Genet. 11, 499-505.

(23) Rolf, B., Bastmeyer, M., Schachner, M. and Bartsch, U. (2002). Pathfinding errors of corticospinal axons in neural cell adhesion molecule-deficient mice. J. Neurosci. 22, 8357-8362.

(24) Senn, C., Kutsche, M., Saghatelyan, A., Bosl, M., Lohler, J., Bartsch, U., Morellini, F. and Schachner, M. (2002). Mice deficient for the HNK-1 sulfotransferase show alterations in synaptic efficacy and spatial learning and memory. Mol. Cell. Neurosci. 20, 712-729.

(25) Simon-Haldi, M., Mantei, N., Franke, J., Voshol, H. and Schachner, M. (2002). Identification of a peptide mimic of the L2/HNK-1 carbohydrate epitope. J. Neurochem. 83,1380-1388.

(26) Stolt, C.C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U. and Wegner, M. (2002). Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes Dev. 16, 165-170.

(27) Strekalova, T., Sun, M., Sibbe, M., Evers, M., Dityatev, A., Gass, P. and Schachner, M. (2002). Fibronectin domains of extracellular matrix molecule tenascin-C modulate hippocampal learning and synaptic plasticity. Mol. Cell. Neurosci. 21, 173-187.

(28) Sytnyk, V., Leshchyns'ka, I., Delling, M., Dityateva, G., Dityatev, A. and Schachner, M. (2002). Neural cell adhesion molecule promotes accumulation of TGN organelles at sites of neuron-to-neuron contacts. J. Cell Biol. 159, 649-661.

(29) Thies, A., Schachner, M., Moll, I., Berger, J., Schulze, H., Brunner, G. and Schumacher, U. (2002).

Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma. Eur. J. Cancer 38, 1708-1716.

(30) Venero, C., Tilling, T., Hermans-Borgmeyer, I., Schmidt, R., Schachner, M. and Sandi, C. (2002). Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neuroscience 115, 1211-1219.

(31) Vyas, A.A., Patel, H.V., Fromholt, S.E., Heffer-Lauc, M., Vyas, K.A., Dang, J., Schachner, M. and Schnaar, R.L. (2002). Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. Proc. Natl. Acad. Sci. USA 99, 8412-8417.

(32) Becker, C.G., Schweitzer, J., Feldner, J., Becker, T. and Schachner, M. (2003). Tenascin-R as a repellent guidance molecule for developing optic axons in zebrafish. J. Neurosci. 23, 6232-6237.

(33) Becker, T., Bossenz, M., Tursun, B., Schluter, A., Peters, M.A., Becker, C.G., Ostendorff, H.P. and Bach, I. (2003). Comparing protein stabilities during zebrafish embryogenesis. Methods Cell Sci. 25, 85-89.

(34) Becker, T., McLane, M.A. and Becker, C.G. (2003). Integrin antagonists affect growth and pathfinding of ventral motor nerves in the trunk of embryonic zebrafish. Mol. Cell. Neurosci. 23, 54-68.

(35) Bruckner, G., Grosche, J., Hartlage-Rubsamen, M., Schmidt, S. and Schachner, M. (2003). Region and lamina-specific distribution of extracellular matrix proteoglycans, hyaluronan and tenascin-R in the mouse hippocampal formation. J. Chem. Neuroanat. 26, 37-50.

(36) Buhusi, M., Midkiff, B.R., Gates, A.M., Richter, M., Schachner, M. and Maness, P.F. (2003). Close homolog of L1 is an enhancer of integrin-mediated cell migration. J. Biol. Chem. 278, 25024-25031.

(37) Chaisuksunt, V., Campbell, G., Zhang, Y., Schachner, M., Lieberman, A.R. and Anderson, P.N. (2003). Expression of regeneration-related molecules in injured and regenerating striatal and nigral neurons. J. Neurocytol. 32, 161-183.

(38) Chen, S., Mange, A., Dong, L., Lehmann, S. and Schachner, M. (2003). Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival. Mol. Cell. Neurosci. 22, 227-233.

(39) Dihne, M., Bernreuther, C., Sibbe, M., Paulus, W. and Schachner, M. (2003). A new role for the cell adhesion molecule L1 in neural precursor cell proliferation, differentiation, and transmitter-specific subtype generation. J. Neurosci. 23, 6638-6650.

(40) Dityatev, A.E., Altinbaev, R.Sh., Astrelin, A.V. and Voronin L. (2003). Combining principal component and spectral analyses with the method of moments in studies of quantal transmission. J. Neurosci. Methods 130, 173-199. Special issue: Adapting Quantal Analysis to Central Synapses -Edited by D.S. Faber and S.J. Redman.

(41)Dityatev, A. and Schachner, M. (2003). Extracellular matrix molecules and synaptic plasticity. Nat. Rev. Neurosci. 4, 456-468.

(42)Dong, L., Chen, S., Bartsch, U. and Schachner, M. (2003). Generation of affinity matured scFv antibodies against mouse neural cell adhesion molecule L1 by phage display. Biochem. Biophys. Res. Commun. 301, 60-70.

(43)Dong, L., Chen, S. and Schachner, M. (2003). Single chain Fv antibodies against neural cell adhesion molecule L1 trigger L1 functions in cultured neurons. Mol. Cell. Neurosci. 22, 234-247.

(44)Freitag, S., Schachner, M. and Morellini, F. (2003). Behavioral alterations in mice deficient for the extracellular matrix glycoprotein tenascin-R. Behav. Brain Res. 145, 189-207.

(45)Frints, S.G., Marynen, P., Hartmann, D., Fryns, J.P., Steyaert, J., Schachner, M., Rolf, B., Craessaerts, K., Snellinx, A., Hollanders, K., D'Hooge, R., De Deyn, P.P. and Froyen, G. (2003). CALL interrupted in a patient with non-specific mental retardation: gene dosage-dependent alteration of murine brain development and behavior. Hum. Mol. Genet. 12, 1463-1474.

(46)Fux, C.M., Krug, M., Dityatev, A., Schuster, T. and Schachner, M. (2003). NCAM180 and glutamate receptor subtypes in potentiated spine synapses: an immunogold electron microscopic study. Mol. Cell. Neurosci. 24, 939-950.

(47)Heller, M., von der Ohe, M., Kleene, R., Mohajeri, M.H. and Schachner, M. (2003). The immunoglobulin-superfamily molecule basigin is a binding protein for oligomannosidic carbohydrates: an anti-idiotypic approach. J. Neurochem. 84, 557-565.

(48)Hu, Q.D., Ang, B.T., Karsak, M., Hu, W.P., Cui, X.Y., Duka, T., Takeda, Y., Chia, W., Sankar, N., Ng, Y.K., Ling, E.A., Maciag, T., Small, D., Trifonova, R., Kopan, R., Okano, H., Nakafuku, M., Chiba, S., Hirai, H., Aster, J.C., Schachner, M., Pallen, C.J., Watanabe, K. and Xiao, Z.C. (2003). F3/Contactin acts as a functional ligand for notch during oligodendrocyte maturation. Cell 115, 163-175.

(49)Kalus, I., Schnegelsberg, B., Seidah, N.G., Kleene, R. and Schachner, M. (2003). The proprotein convertase PC5A and a metalloprotease are involved in the proteolytic processing of the neural adhesion molecule L1. J. Biol. Chem. 278, 10381-10388.

(50)Law, J.W., Lee, A.Y., Sun, M., Nikonenko, A.G., Chung, S.K., Dityatev, A., Schachner, M. and Morellini, F. (2003). Decreased anxiety, altered place learning, and increased CA1 basal excitatory synaptic transmission in mice with conditional ablation of the neural cell adhesion molecule L1. J. Neurosci. 23, 10419-10432.

(51)Leshchyns'ka, I., Sytnyk, V., Morrow, J.S. and Schachner, M. (2003). Neural cell adhesion molecule (NCAM) association with PKC{beta}2 via {beta}I spectrin is implicated in NCAM-mediated neurite outgrowth. J. Cell Biol. 161, 625-639.

(52)Lieberoth, B.C., Becker, C.G. and Becker, T. (2003). Double labeling of neurons by retrograde axonal tracing and non-radioactive in situ hybridization in the CNS of adult zebrafish. Methods Cell Sci. 25, 65-70.

(53)Mears, S., Schachner, M. and Brushart, T.M. (2003). Antibodies to myelin-associated glycoprotein accelerate preferential motor reinnervation. J. Peripher. Nerv. Syst. 8, 91-99.

(54)Miyamoto, K., Miyake, S., Schachner, M. and Yamamura, T. (2003). Heterozygous null mutation of myelin P0 protein enhances susceptibility to autoimmune neuritis targeting P0 peptide. Eur. J. Immunol. 33, 656-665.

(55)Montag-Sallaz, M., Montag, D. and Schachner, M. (2003). Altered processing of novel information in N-CAM-deficient mice. Neuroreport 14, 1343-1346.

(56)Neidhardt, J., Fehr, S., Kutsche, M., Lohler, J. and Schachner, M. (2003). Tenascin-N: characterization of a novel member of the tenascin family that mediates neurite repulsion from hippocampal explants. Mol. Cell. Neurosci. 23, 193-209.

(57)Nie, D.Y., Zhou, Z.H., Ang, B.T., Teng, F.Y., Xu, G., Xiang, T., Wang, C.Y., Zeng, L., Takeda, Y., Xu, T.L., Ng, Y.K., Faivre-Sarrailh, C., Popko, B., Ling, E.A., Schachner, M., Watanabe, K., Pallen, C.J., Tang, B.L. and Xiao Z.C. (2003). Nogo-A at CNS paranodes is a ligand of Caspr: possible regulation of K(+) channel localization. EMBO J. 22, 5666-5678.

(58)Nikonenko, A., Schmidt, S., Skibo, G., Bruckner, G. and Schachner, M. (2003). Tenascin-R-deficient mice show structural alterations of symmetric perisomatic synapses in the CA1 region of the hippocampus. J. Comp. Neurol. 456, 338-349.

(59)Pratte, M., Rougon, G., Schachner, M. and Jamon, M. (2003). Mice deficient for the close homologue of the neural adhesion cell L1 (CHL1) display alterations in emotional reactivity and motor coordination. Behav. Brain Res. 147, 31-39.

(60)Putthoff, P., Akyuz, N., Kutsche, M., Zardi, L., Borgmeyer, U. and Schachner, M. (2003). Structure of the murine tenascin-R gene and functional characterisation of the promoter. Biochem. Biophys. Res. Commun. 308, 940-949.

(61)Rolf, B., Lang, D., Hillenbrand, R., Richter, M., Schachner, M. and Bartsch, U. (2003). Altered expression of CHL1 by glial cells in response to optic nerve injury and intravitreal application of fibroblast growth factor-2. J. Neurosci. Res. 71, 835-843.

(62)Roonprapunt, C., Huang, W., Grill, R., Friedlander, D., Grumet, M., Chen, S., Schachner, M. and Young, W. (2003). Soluble cell adhesion molecule L1-Fc promotes locomotor recovery in rats after spinal cord injury. J. Neurotrauma 20, 871-882.

(63)Rünker, A.E., Bartsch, U., Nave, K.A. and Schachner, M. (2003). The C264Y missense mutation in the extracellular domain of L1 impairs protein trafficking in vitro and in vivo. J. Neurosci. 23, 277-286.

(64) Saghatelyan, A.K., Snapyan, M., Gorissen, S., Meigel, I., Mosbacher, J., Kaupmann, K., Bettler, B., Kornilov, A.V., Nifantiev, N.E., Sakanyan, V., Schachner, M. and Dityatev, A. (2003). Recognition molecule associated carbohydrate inhibits postsynaptic GABA(B) receptors: a mechanism for homeostatic regulation of GABA release in perisomatic synapses. Mol. Cell. Neurosci. 24, 271-282.

(65) Schweitzer, J., Becker, T., Becker, C.G. and Schachner, M. (2003). Expression of protein zero is increased in lesioned axon pathways in the central nervous system of adult zebrafish. Glia 41, 301-317.

(66) Senner, V., Schmidtpeter, S., Braune, S., Puttmann, S., Thanos, S., Bartsch, U., Schachner, M. and Paulus, W. (2003). AMOG/beta2 and glioma invasion: does loss of AMOG make tumour cells run amok? Neuropathol. Appl. Neurobiol. 29, 370-377.

(67) Tang, J., Wagner, S., Schachner, M., Dityatev, A. and Wotjak, C.T. (2003). Potentiation of amygdaloid and hippocampal auditory-evoked potentials in a discriminatory fear-conditioning task in mice as a function of tone pattern and context. Eur. J. Neurosci. 18, 639-650.

(68) Thelin, J., Waldenstrom, A., Bartsch, U., Schachner, M. and Schouenborg, J. (2003). Heat nociception is severely reduced in a mutant mouse deficient for the L1 adhesion molecule. Brain Res. 965, 75-82.

(69) Wuhrer, M., Geyer, H., von der Ohe, M., Gerardy-Schahn, R., Schachner, M. and Geyer, R. (2003). Localization of defined carbohydrate epitopes in bovine polysialylated NCAM. Biochimie 85, 207-218.

(70) Albach, C., Damoc, E., Denzinger, T., Schachner, M., Przybylski, M. and Schmitz, B. (2004). Identification of N-glycosylation sites of the murine neural cell adhesion molecule NCAM by MALDI-TOF and MALDI-FTICR mass spectrometry. Anal. Bioanal. Chem. 378, 1129-1135.

(71) Brenneke, F., Bukalo, O., Dityatev, A. and Lie, A.A. (2004). Mice deficient for the extracellular matrix glycoprotein tenascin-R show physiological and structural hallmarks of increased hippocampal excitability, but no increased susceptibility to seizures in the pilocarpine model of epilepsy. Neuroscience 124, 841-855.

(72) Bukalo, O., Fentrop, N., Lee, A.Y., Salmen, B., Law, J.W., Wotjak, C.T., Schweizer, M., Dityatev, A. and Schachner, M. (2004). Conditional ablation of the neural cell adhesion molecule reduces precision of spatial learning, long-term potentiation, and depression in the CA1 subfield of mouse hippocampus.J. Neurosci. 24, 1565-1577.

(73) Gurevicius, K., Gureviciene, I., Valjakka, A., Schachner, M., and Tanila,H. (2004). Enhanced cortical and hippocampal neuronal excitability in mice deficient in the extracellular matrix glycoprotein tenascin-R. Mol. Cell. Neurosci. 25, 515-523.

(74) Kleene, R. and Schachner, M. (2004). Glycans and neural cell interactions. Nat. Rev. Neurosci. 5, 195-208.

(75) Naus, S., Richter, M., Wildeboer, D., Schachner, M., Moss, M. and Bartsch, J.W. (2004). Ectodomain shedding of the neural recognition molecule CHL1 by the metalloprotease-disintegrin ADAM8 promotes neurite outgrowth and suppresses neuronal cell death. J. Biol. Chem. 279, 16083-16090.

(76) Ohyama, K., Tan-Takeuchi, K., Kutsche, M., Schachner, M., Uyemura, K., and Kawamura, K. (2004). Neural cell adhesion molecule L1 is required for fasciculation and routing of thalamocortical fibres and corticothalamic fibres. Neurosci. Res. 48, 471-475.

(77) Saghatelyan, A., De Chevigny, A., Schachner, M., and Lledo, P.M. (2004). Tenascin-R mediates activity-dependent recruitment of neuroblasts in the adult mouse forebrain. Nat. Neurosci. 7, 347-356.

(78) Sytnyk, V., Leshchyns'ka, I., Dityatev, A. and Schachner, M. (2004). Trans-Golgi network delivery of synaptic proteins in synaptogenesis. J. Cell Sci. 117, 381-388.

(79) Venero, C., Tilling, T., Hermans-Borgmeyer, I., Herrero, A.I., Schachner, M. and Sandi, C. (2004). Water maze learning and forebrain mRNA expression of the neural cell adhesion molecule L1. J. Neurosci. Res. 75, 172-181.

(80) Wiencken-Barger, A.E., Mavity-Hudson, J., Bartsch, U., Schachner, M. and Casagrande, V.A. (2004). The role of L1 in axon pathfinding and fasciculation. Cereb. Cortex 14, 121-131.

Theses

Diploma

Henning, Stefan (2002). Inhibition der Apoptose durch Proteine der L1-Familie und Stickstoffmonoxid. Fachhochschule Hamburg-Bergedorf.

Meier, Ingo (2002). Partielle Klonierung und Analyse der Expressionsmuster neuraler Zelladhäsionsmoleküle im Zebrafisch, *Danio rerio*. Fachhochschule Hamburg-Bergedorf.

Kaizik, Stefan (2003). Identifikation extrazellulärer Bindungspartner des neuralen Zelladhäsionsmoleküls CHL1. Universität Hamburg.

Novak, Daniel (2003). Untersuchungen zur potentiellen Interaktion zwischen MUPP-1 und NCAM. Universität Hamburg.

Seier, Anne (2003). Klonierung und Expression des humanen Zellerkennungsmoleküls L1 in *Pichia pastoris*. Fachhochschule Hamburg-Bergedorf.

Köhlitz, Isabel (2004). Expression und Reinigung des Zellerkennungsmoleküls mL1 in *Pichia pastoris*. Fachhochschule Hamburg-Bergedorf.

Dissertations (PhD)

Albert, Martine (2001). Isolierung und Charakterisierung rekombinanter antiidiotypischer Antikörperfragmente gegen den Kohlenhydrat-erkennenden monoklonalen Antikörper HNK-1. Universität Stuttgart.

Bormann, Ulrich (2001). Yeast two-hybrid Systeme zur Identifikation zytoplasmatischer Interaktionspartner der neuralen Zelladhäsionsmoleküle MAG, P0 und NCAM. Universität Hamburg.

Delling, Markus (2001). Regulation of G protein-activated inwardly rectifying potassium channels by the neural cell adhesion molecule NCAM. Universität Hamburg.

Evers, Matthias R. (2001). Generation and phenotyping of tenascin-C deficient mice and cloning and characterization of new members of the HNK-1 family of sulfotransferases. Ruhr-Universität Bochum.

Karsak, Meliha (2001). Untersuchung der differenziellen Genexpression in sensorischen und motorischen Nervenästen des peripheren Nervensystems. Universität Hannover.

Neidhardt, John (2001). Identification, cloning and characterization of the novel tenascin family member tenascin-N and conditional gene targeting of tenascin-R. Universität Hannover.

Schnegelsberg, Birthe (2001). In vitro Untersuchungen zur posttranslationalen Prozessierung der Zelladhäsionsmoleküle L1 und CHL1 und Charakterisierung eines neuen Zytoskelett-assoziierten Proteins: Protein CC als Linkerprotein zwischen dem Zelladhäsionsmolekül NCAM und dem Zytoskelett. Universität Hamburg.

Sytnyk, Vladimir (2001). Trafficking of cell adhesion molecules in growing neurites. National Academy of Sciences of Ukraine, Kiev, Ukraine.

Bukalo, Olena (2002). The roles of extracellular matrix molecules in synaptic transmission and plasticity in the mouse hippocampus. Universität Hamburg.

Gimnopoulos, Dimitrios (2002). Funktionelle Analyse von Zellerkennungsmolekülen der Immunglobulin-Superfamilie während der Entwicklung und Regeneration des Nervensystems des Zebrafisches *Danio rerio* (HAMILTON, 1822). Universität Hamburg.

Richter, Melanie (2002). Identification and characterization of intracellular binding partners of CHL1 (close homologue of L1) neural cell recognition molecule. Universität Bielefeld.

Rolf, Bettina (2002). Functional analysis of cell adhesion molecules L1, CHL1 and NCAM in vivo. Universität Bielefeld.

Rünker, Annette (2002). Mutations in the extracellular domain of the neural cell adhesion molecule L1 impair protein trafficking in vitro and in vivo. Universität Bielefeld.

Salmen, Benedikt (2002). Alterations of Ca2+ mediated signaling in tenascin-C and NCAM deficient mice. Ruhr-Universität Bochum.

Wagner, Stefanie (2002). Untersuchung des Verhaltens von C57BL/6J-Mäusen in einem passiven Vermeidetest mit anschließender Analyse der Genexpression neuraler Zelladhäsionsmoleküle. Universität Hannover.

Schweitzer, Jörn (2003). Klonierung und funktionelle Analyse der Zellerkennungsmoleküle Tenascin-R, Tenascin-C und P0 in Entwicklung und Regeneration im Zebrafisch *Danio rerio* (Hamilton 1822). Universität Hamburg.

Strekalova, Helen (2003). Alzheimer disease: Identification and characterization of putative binding partners of Amyloid Precursor Protein (APP) and cell adhesion molecules as biochemical markers. Universität Hamburg.

Wesche, Kai-Oliver (2003). Analysis of dysregulated molecules in the nervous system of tenascin-R deficient mice. Universität Hamburg.

Medical Dissertations

Cangür, Hakan (2003). Die Konstruktion zweier Gensonden für das Neurale Zelladhäsionsmolekül NCAM und die HNK-1-Sulfotransferase. Universität Hamburg.

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Habilitation

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Institut für Entwicklungsneurobiologie

H. Chica Schaller

In early development of the nervous system intercellular communication is important for establishing the structure of the brain and the pattern of synaptic contacts. The main aim of our research is to find out how extracellular signals are transmitted over appropriate receptors to the interior of the cell to regulate proliferation, differentiation, pattern formation, and behavior.

In the report period we concentrated on characterizing three classes of molecules, heptahelical G-protein-coupled receptors (GPCRs), vacuolar protein sorting (VPS)10-domain containing receptors, and nuclear receptors.

GPCRs are the largest family of cell-surface transmembrane receptors, encoded by more than 1,000 genes in the human genome. At present 200 GPCRs, for which ligands are known, and approximately 150 ”orphan” GPCRs, for which ligands remain to be identified, are found in the public database. This excludes the odorant, vomeronasal, and taste receptors. Finding new GPCRs and new ligands will contribute to a better understanding of the normal functions of these receptors and the roles they play in disease. GPCRs and their ligands are of special interest for pharmacology, since they are targets for 40-50% of all present drugs. In recent years we discovered 13 new GPCRs and analyzed their distribution in various organs and during development. For deorphanization we chose GPCRs with either an interesting expression pattern or with homology to peptide receptors. We succeeded to find ligands for five orphan GPCRs, two of our novel GPCRs were deorphanized by others.

VPS10-domain containing receptors are of special importance in early brain development, since they function both as chaperons in intracellular trafficking and as receptors or coreceptors for extracellular ligands. A typical example is sortilin, also known as third neurotensin receptor. It helps to fold and transport secretory proteins from the ER and Golgi to the extracellular membrane, but also functions as receptor or, in shed form, as binding protein for the neuropeptide neurotensin. As coreceptor for the neurotrophin receptor p75 it binds pro-NGF to induce apoptosis in nerve cells. A similar, more complicated function is postulated for SorLA/LR11, which we characterized, both in its membrane and shed form, as head-activator (HA) binding protein. Due to the presence of lipoprotein binding domains, it also interacts with apolipoprotein E. Since SorLA/LR11 is downregulated in brains of Alzheimer patients, a role in disease pathology is likely.

As new members of the VPS10-domain containing receptor family we discovered SorCS1-3, which seem to be important for memory consolidation.

In an independent research project U. Borgmeyer continued his studies on nuclear receptors and their effects on transcriptional control in early mammalian development. With the guest group of A. Methner we share a common interest in GPCRs and the technologies to study them.

1. Deorphanization of GPR6 and GPR12 as lysophospholipid receptors

Atanas Ignatov, Julia Lintzel, Irm Hermans-Borgmeyer

The deorphanization of GPCRs was based on two major components: phylogenetic analysis and improved cell-surface expression of the receptor, the latter enhanced by including an artificial signal peptide. For ligand screening several assays were used, including an aequorin-based bioluminescence assay, expression in frog oocytes (collaboration with H.-J. Kreienkamp), a luciferase receptor gene assay, and internalization after ligand binding. A detailed phylogenetic analysis, contributed by Patrick Joost of Methner's group, showed that GPR3, GPR6, and GPR12 are closely related to the melanocortin-like peptide family, but also to phospholipid and cannabinoid receptors. Independent of the expression system used, two lysophospholipids were identified acting at nanomolar concentrations as ligands for GPR6 and GPR12, sphingosine-1-phosphate (S1P) and the related lysophospholipid, sphingosylphosphorylcholine (SPC), respectively. The G-protein coupling of GPR6 and GPR12 was also investigated. The increase in potassium currents in frog oocytes was completely inhibited by pertussis toxin, suggesting coupling of both receptors to an inhibitory G protein. In CHO cells, transfected with GPR6, the S1P-induced calcium mobilization was abolished by the sphingosine-kinase inhibitor, DL-threo-dihydrosphingosine. This was taken as evidence that GPR6 activates Ca^{2+} over the sphingosine-kinase pathway (Fig. 1).

To get insight into possible functions of GPR6 and GPR12, their expression was studied. Northern blot and reverse

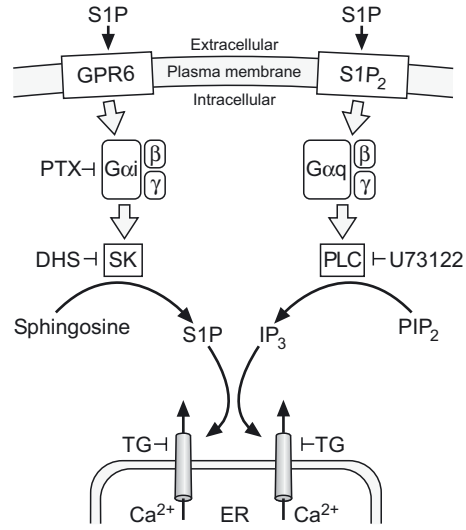


Figure 1. Model for S1P-stimulated signal-transduction pathways in CHO cells. The scheme depicts the most likely signaling pathways after stimulation of the endogenous S1P receptor ($S1P_2$) and GPR6 with S1P. *ER*, endoplasmic reticulum; *GPR6*, G protein-coupled receptor 6; *IP_3*, inositol 1,4,5-trisphosphate; *PIP2*, phosphatidylinositol 4,5-biphosphate; *PLC*, phospholipase C; *PTX*, pertussis toxin; *S1P*, sphingosine-1-phosphate; *SK*, sphingosine kinase; *TG*, thapsigargin; *U73122*, phospholipase C inhibitor.

transcription polymerase chain reaction analysis revealed predominant expression of GPR6 and GPR12 in the brain. To investigate the distribution of GPR12 during mouse embryonal development and in adult mouse brain, in situ hybridization was performed. Highest signal intensities of GPR12 mRNA were detected in the cerebral cortex and the hippocampus. In the mature mouse brain, GPR12 was predominantly expressed in the limbic system (Fig. 2). Based on the distribution of GPR12 in the embryonal mouse brain, we investigated its role in cultures of embryonal cerebral

cortical neurons. Treatment of these neurons with SPC led to an earlier expression of synaptophysin and to an increase in synaptic contacts (collaboration with A. Methner's group).

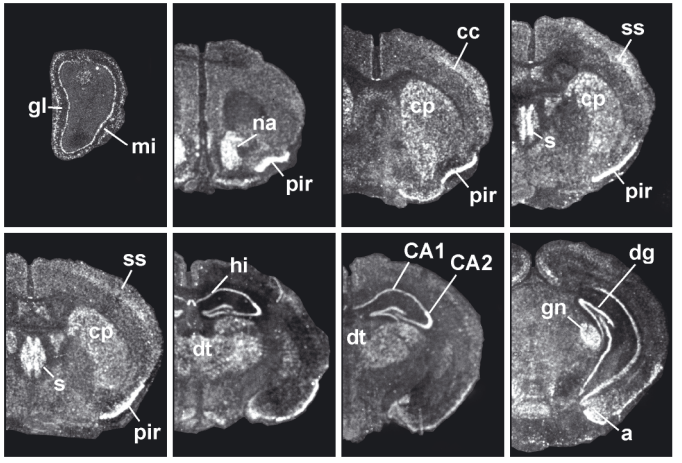


Figure 2. Expression of GPR12 in distinct areas of the adult CNS. Dark-field photomicrographs of photoemulsion-dipped coronal sections through an adult mouse forebrain and midbrain are shown, arranged in rostral to caudal direction. *a*, amygdala; *CA1*, *CA2* layers of hippocampus; *cc*, cerebral cortex; *cp*, caudate putamen; *dg*, dentate gyrus; *dt*, dorsal thalamus; *gl*, glomerular layer of olfactory bulb; *gn*, geniculate nucleus; *hi*, hippocampus; *mi*, mitral cell layer of olfactory bulb; *na*, nucleus acumbens; *pir*, piriform cortex; *s*, septum; *ss*, somatosensory cortex

Since sphingosine kinase plays an important role in cell survival, the involvement of GPR6 in this process was assessed. CHO cells cultured in serum-free medium or treated with hydroxyl peroxide undergo cell death, which could be prevented by transfection of cells with GPR6 and treatment with S1P. The survival-promoting effect of GPR6 was inhibited by DL-threo-dihydrosphingosine, suggesting the important role of sphingosine kinase in the S1P/GPR6-survival mechanism.

2. Discovery of two orphan GPCRs as receptors for HA

Meriem Rezgaoui, Ute Süsens, Inga Franke, Kai-Oliver Wesche

We found that in all cell lines, which react with HA, two GPCRs were expressed with homology to endothelin, bombesin, and neuromedin receptors. Like HA these peptides influence cell proliferation.

Human GPCR clones and antibodies were obtained in a collaborative project from the group of R. Takahashi, Japan, who had found that one of the GPCRs requires parkin for proper folding and processing. In patiens with Parkinson's disease the misfolded receptor accumulates in the ER of cells in the substantia nigra and contributes to cell death.

The two receptors share 51% homology with each other, which was a first indication that they may share a common ligand. We found that HA is able to react with both receptors starting at subnanomolar concentrations. In collaboration with G. Glassmeier and J.R. Schwarz of the Institute of Physiology (UKE) we found that treatment of COS-7 cells, expressing these receptors, with HA leads to an increase of membrane currents. The effect is blockable by pertussis toxin, hinting at an inhibitory G protein for signal transduction. Both in transiently transfected COS-7 cells and in stably transfected HEK cells, HA induced internalization of the receptors and stimulated mitosis and cell proliferation. FRET analysis revealed direct binding of HA to the receptors. This was confirmed by studies with a Cy3-labeled HA analogue, which bound better to cells overexpressing the receptors.

3. Deorphanization of GPR100 as novel bradykinin receptor

Katrin Boels

Full-length GPR100 was amplified from a cDNA library of the neuroendocrine cell line BON, which is derived from a human pancreas carcinoid. The open reading frame, present on a single exon, coded for a protein of 374 amino acids. Sequence comparison revealed a 43% identity and 59% similarity at the amino acid level with the human orphan somatostatin and angiotensin-like peptide receptor (SALPR). Stable expression of GPR100 in CHO cells revealed bradykinin and kallidin as effectors for calcium mobilization. Dose-response curves yielded EC_{50} values for both ligands in the low nanomolar range, while the respective analogues without arginine at the carboxy terminus were inactive (Fig. 3). Calcium mobilization was inhibited by the phospholipase C blocker U73122, but not by pertussis toxin, suggesting involvement of the G-protein subunit αq in signal transduction.

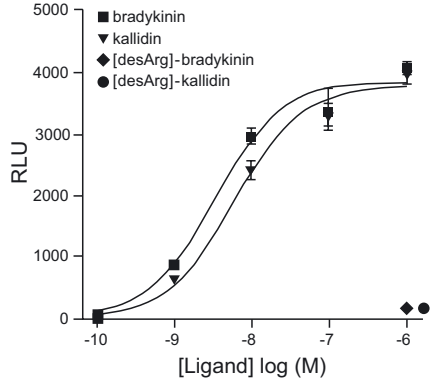


Figure 3. Bradykinin and kallidin are high-affinity ligands for GPR100. CHO cells containing Ga16 and apoaequorin, stably expressing GPR100 were treated with increasing concentrations of ligands. Ca^{2+}-induced bioluminescence was measured at 469 nm, and expressed as relative light units (RLUs), from which the basal response to the injection stimulus was substracted.

In line with the main function of kinins as peripheral hormones, we found that GPR100 was expressed predominantly in peripheral tissues. In contrast to GPR100 we found that SALPR showed a distinct pattern of expression in defined areas of the central nervous system. Given the high homology between GPR100 and SALPR we assume that SALPR is a new kinin receptor, present in the central nervous system.

4. GalRL, GPR19, TRHRL1, TRHRL2, GPR99, and DEZ

Atanas Ignatov, Sabine Hoffmeister-Ullerich, Ute Süsens, Jens Urny, Irm Hermans-Borgmeyer

GalRL. GalRL shows 25-26% identity and 41-43% similarity at the amino-acid level with the galanin-receptor subfamily. We found that micromolar concentrations of galanin were required to increase Ca^{2+} mobilization in CHO cells transiently expressing GalRL, which suggests a peptide similar to galanin as cognate ligand for this receptor.

In situ hybridization analysis demonstrated a unique restricted expression of GalRL in the habenular complex (Fig. 4). The latter represents a major component of the dorsal diencephalic conduction system, playing a role in autonomic and endocrine regulation, pain processing, motor

and feeding behaviour, and brain reward mechanisms. This hints at an important and perhaps unique role of GalRL in the control of autonomic and behavioral functions.

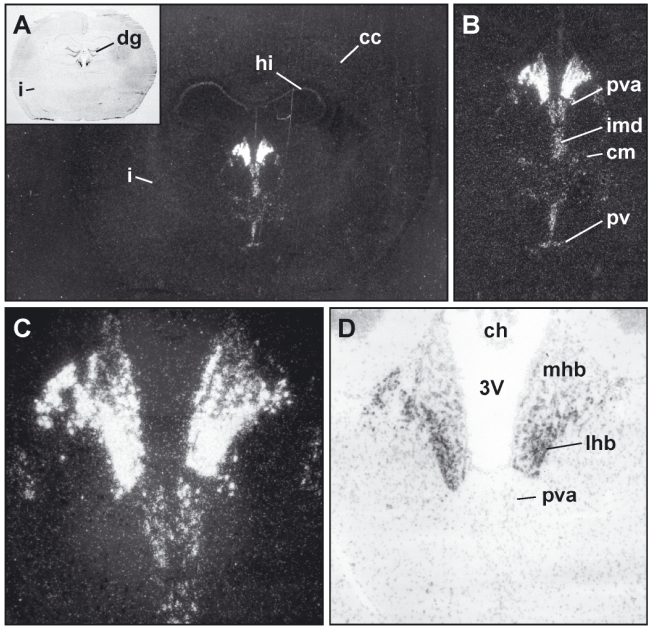


Figure 4. GalRL is expressed in distinct nuclei of the mouse brain. (A-C) Darkfield photomicrographs of frontal sections hybridized to the [^{35}S]-labeled GalRL-specific riboprobe are shown. Hybridization signals appear white. The insert in (A) shows the Giemsa-stained brightfield image of the section presented enlarged as darkfield photograph. Note that the dentate gyrus appears black due to the Giemsa staining, while the habenula accumulates silver grains seen as black spots. (D) is a brightfield photomicrograph corresponding to the darkfield photograph presented in (C). *cc*, cerebral cortex; *ch*, choroids plexus; *cm*, central thalamic nucleus; *dg*, dentate gyrus; *hb*, habenula; *hi*, hippocampus; *imd*, intermediodorsal thalamic nucleus; *lhb*, laterial habenula; *mhb*, median habenula; *pv*, paraventricular nucleus of the hypothalamus; *pva*, paraventricular nucleus of the thalamus; *3V*, third ventricle.

GPR19. GPR19 was identified using an EST clone from a mouse mammary tumor, which contained the full-length sequence. In the adult mouse GPR19 was predominantly expressed in brain and testis. High levels of transcription were present in the olfactory bulb, the hippocampus, hypothalamic nuclei, and the cerebellum. During embryogenesis we detected GPR19 expression at E7.5 in the developing germ layers of the embryo proper. At E8.5 GPR19 transcripts accumulated in the neuroepithelium, and were observed along the entire neural plate. This became more evident two days later, when high amounts of GPR19 mRNA were detectable in the wall of the telencephalic vesicle, the optic cup, and in the neural tube of the trunk region. With ongoing development the signals persisted in areas with dividing cells lining the neural canal and the ventricles. In other regions of the body no prominent hybridization signals of GPR19 were detected during embryogenesis, implying an important role of this receptor in early nervous system development.

TRHRL1 and TRHRL2. Recently, we discovered two new GPCRs with some homology to TRH receptors, hence their names, TRH-like receptor 1 and 2. Both contain an intron at the end of the first transmembrane domain, share 45% homology at the amino acid level, and might react with the same ligand, which, from the phylogenetic relatedness, is most likely a peptide. Since predictions for the first exon were equivocal and no ESTs available, we identified mouse cDNAs by 5' Race. Northern blot analysis showed expression of TRHRL1 in brain and in E15 embryos. In the adult human brain strong signals were present in the caudate nucleus, medulla, and putamen and weaker ones in amygdala, thalamus and spinal cord. With TRHRL2 no signal was seen on Northern blots, but in a mouse cDNA panel heart, spleen, E11 and E15 embryos were positive. This indicates that the

two receptors may serve different functions, TRHRL1 being brain specific, while TRHRL2 may play a role in peripheral organs.

GPR99 and DEZ. Some time ago we cloned GPR99 (W. Hampe) and DEZ (G. Hermey, A. Methner). They were identified by others as nucleotide and chemoattractant receptors, binding to adenosin/AMP and chemerin as endogenous ligands, respectively.

5. VPS10-domain receptors and SPP

Irm Hermans-Borgmeyer, Jens Urny, Meriem Rezgaoui, Björn Riedel, Inga Franke, Moritz Hentschke, Uwe Borgmeyer

SorCS1-3. In recent years we isolated three additional members of the VPS10-domain containing receptors, SorCS1-3, which due to their unique protein structure form a novel subgroup of this sorting-receptor family. They are characterized by the presence of a leucine-rich domain following the VPS10-domain in their extracellular portion. The expression pattern of SorCS2 and SorCS3 in the developing and adult brain was found to be mostly complementary. SorCS1, which exists in four different splice variants of its intracellular region (collaboration with G. Hermey), exhibited a wider distribution in the brain. In some areas it colocalized with SorCS2, in others with SorCS3.

The temporal and spatial appearance of the SorCS genes during development in the nervous system suggested a role in establishing synaptic connectivity in the brain. Therefore, in collaboration with the group of D. Kuhl, we analyzed if the SorCS genes are regulated by electric activity. Upon injection of kainic acid we indeed detected an increase in transcript levels for SorCS1 and SorCS3 in the forebrain, especially the hippocampus. Using cycloheximide injection in parallel to kainic acid stimulation, we demonstrated that the upregulation of SorCS3 transcripts was independent and that of SorCS1 dependent on protein synthesis.

These results prompted us to analyze the promoter regions of the three SorCS genes. We could narrow down the essential promoter region of SorCS2 to a few hundred base pairs. This fragment was then cloned in front of a ß-galactosidase gene and used to generate transgenic mice by pronuclear injection. Analysis of the embryos obtained at different stages of development revealed the same expression pattern as that of the endogenous gene.

SorLA/LR11 in Alzheimer's disease. In a microarray study SorLA/LR11 protein was identified as a transcript down-regulated in sporadic Alzheimer's disease (AD). SorLA/LR11 is enriched in cortical and hippocampal neurons of the human brain. In brains of patients with AD the expression in neurons, but not in astrocytes was reduced. The overall reduction of SorLA/LR11 was confirmed by Western blot analysis (collaboration with A. I. Levey's group).

SorLA/LR11 in addition to HA binds apolipoprotein E (ApoE). Since ApoE interacts with Aβ, a likely scenario is that absence of SorLA/LR11 in neurons contributes to a less efficient removal of Aβ and hence to Alzheimer's pathology. Presence of SorLA/LR11 in stigmoid bodies may support this notion (collaboration with C.-A. Gutekunst).

SPP. In search for HA-binding proteins we screened a human brain cDNA library expressed in the T7 phage display system. Positive was a clone coding for fragments of a protein containing structural features typical for the presenilin-family of aspartate proteases. Full-length cDNAs were isolated from human and mouse brain libraries, coding

for a protein of 377 and 378 amino acids in human and mouse, respectively. This protein was published later by another group as an aspartyl protease that cleaves signal peptides, after signal peptide removal, within the transmembrane domain and was, therefore, named signal-peptide peptidase (SPP).

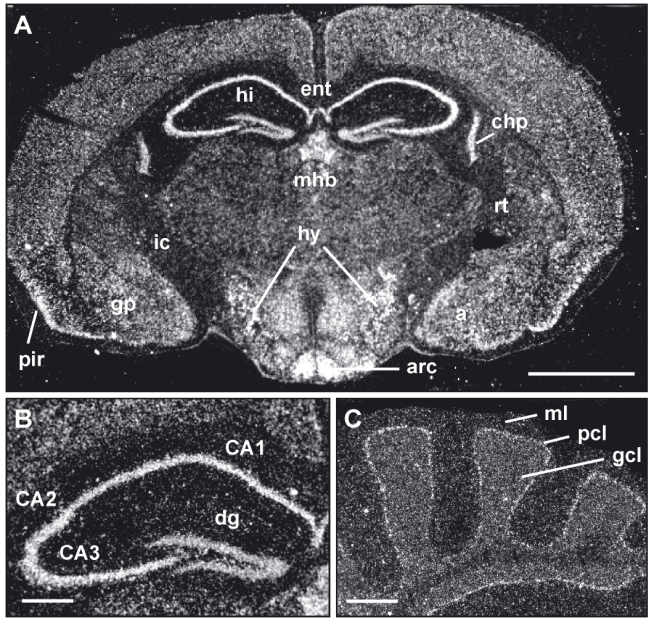


Figure 5. SPP mRNA expression in the adult murine brain. (A) A coronal section of an adult mouse brain at the level of the hippocampus was used to show an overview of SPP expression. (B) A higher magnification of the hippocampal area and (C) of the cerebellum shows that the CA1, CA2, CA3, the dentate gyrus, and the Purkinje cells are strongly labelled, respectively. *a*, amygdala; *arc*, arcuate hypothalamic nucleus; *chp*, choriod plexus; *dg*, dentate gyrus; *ent*, entorhinal cortex; *gcl*, granular cell layer; *gp,* globus pallidus; *hi*, hippocampus; *mhb*, median habenula; *ml*, molecular layer; *pcl*, Purkinje cell layer; *pi*, piriform cortex; *rt*, reticular thalamic nucleus. Scale bars are 400 μm.

Northern blot and in situ hybridization analysis revealed a widespread expression of SPP in many tissues. A distinct pattern of expression in the mature murine brain (Fig. 5) and during development indicated that SPP may play a role in the establishment and maintenance of the nervous system.

We prepared an antiserum against the carboxy-terminal domain of SPP and used it to demonstrate that SPP is oriented in the membrane of the endoplasmic reticulum with its carboxy-terminal tail extending into the cytosol. We also isolated an isoform, which was predominantly expressed at the cell surface.

6. Orphan nuclear receptor signaling

Uwe Borgmeyer, Moritz Hentschke, Eray Gökkurt, Ute Süsens, Irm Hermans-Borgmeyer

Multicellular organisms respond to many extracellular signals by transcriptional activation within a target cell. One of the pathways taken is via intracellular receptors, which constitute the largest superfamily of eukaryotic transcription factors. In response to small ligands they bind to specific genomic response elements and exert transcriptional regulation over development, differentiation and homeostasis. For several novel nuclear receptors the ligands are unknown. The latest isolated mammalian orphan nuclear receptor is the estrogen receptor-related receptor ERRγ. Our expression analysis during development and in the mature mouse brain suggested a function of ERRγ both for neuronal development and maintenance of the adult CNS.

ERRγ binds as a homodimer to derivatives of the direct repeat 5'-AGGTCA-3' and to estrogen response elements. Two modules located in the hinge region and in the ligand binding

domain mediate DNA-independent dimerization. Interestingly, DNA binding requires additional cellular factors that target a small element in the hinge region. Our in vitro experiments demonstrated a Ca^{2+}-dependent interaction with calmodulin. The Ca^{2+}-influx-dependent ERRγ-mediated transcriptional activation led us to propose a new mechanism of ligand-independent activation.

Nuclear receptors recruit specific cofactors to activate or to repress transcription. The spatial expression pattern of ERRγ revealed striking similarities to PGC-1α and PGC1-β. Upon cotransfection both cofactors stimulated the transcriptional activation by ERRγ. The critical role of PGC-1α in regulating multiple aspects of energy suggests a future direction for functional studies. We have identified a unique ERRγ isoform-specific transcriptional activation function (AF-1). By screening a phage display library for human AF-1 binding proteins we have isolated PNRC2, a nuclear receptor cofactor and TLE1, a corepressor. Surprisingly, not only PNRC2, but also TLE1, functioned as ERRγ coactivator. The different cofactors will help to further analyze the molecular events that result in gene transcriptional activation. We are currently investigating how different promoter usage and alternative splicing contribute to the complex expression pattern. In addition, we are generating genetic models to identify target genes and get new insights into the biological functions of ERRγ.

Guest group "Protective Signaling"

Axel Methner

We investigate the molecular mechanisms of neuroprotection in mainly two paradigms of neurological disease: Resistance against oxidative glutamate toxicity as a tool to study protection of neurons against oxidative stress and the phenomenon of ischemic preconditioning to study protection from brain ischemia.

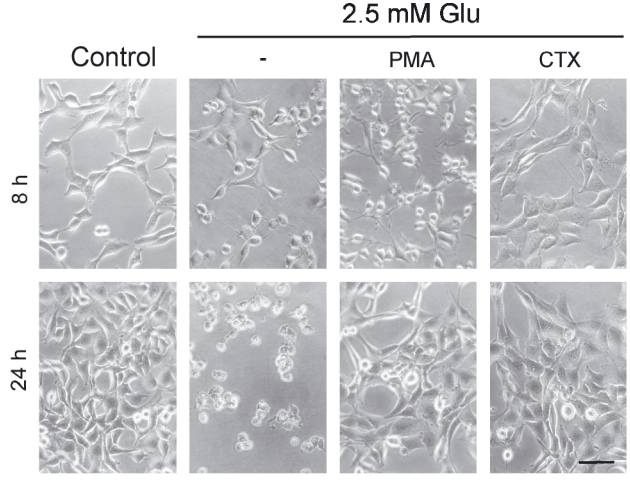
1. Resistance against oxidative stress.

Jan Lewerenz, Patrick Joost, Jan Wessig, Anna Pantlen, Julia Letz, Mert Sahin

Oxidative stress plays an important role in diverse neurological diseases like Alzheimer's and Parkinson's disease. Oxidative glutamate toxicity in the hippocampal cell line HT22 is a model for neuronal death due to oxidative stress, in which extracellular glutamate blocks the glutamate/cystine-antiporter system X_c^-. This leads to depletion of cysteine, a building block of the antioxidant glutathione, subsequent glutathione depletion, accumulation of reactive oxygen species, and programmed cell death.

G-protein signaling. Investigating the role of heterotrimeric G-protein signaling in oxidative glutamate toxicity, we could identify a new pathway, through which the stimulatory G-protein G_s protects neuronal cells against oxidative stress. The protection is mediated by cAMP and associated by an increase in cellular glutathione. It is as powerful as the previously described pathway via G_q initiated phorbol 12-myristate 13-acetate (Fig 1). To identify G-protein coupled receptors (GPCRs) that are involved in these protective pathways, we selected HT22 cells that are resistant against oxidative glutamate toxicity. These showed transcriptional upregulation of the vasointestinal peptide receptor 2 and the metabotropic glutamate receptor 1. Both proved to mediate protection when studied pharmacologically. Thus, we

extended the expression profiling to orphan GPCRs. This led to the identification of one upregulated orphan GPCR that mediates robust protection against glutamate toxicity when overexpressed.



G-protein coupled signaling pathways protect HT22 from oxidative glutamate toxicity. HT22 cells were treated with 2.5 mM glutamate for eight hours with or without addition of either G_q activator phorbol 12-myristate 13-acetate (PMA) added simultaneously with glutamate or the G_s activator cholera toxin (CTX) added 16 hours prior glutamate.

Transcriptional changes that mediate resistance against oxidative stress. A combined candidate and subtractive suppression hybridization approach identified additional transcripts upregulated in glutamate resistance. xCT, the specific subunit of the system X_c^-, was most prominently upregulated. In addition, xCT overexpression proved to be protective. Functional analysis of the system X_c^- in resistant HT22 cells also showed that its inhibition by glutamate is shifted to higher glutamate concentrations. This is most possibly accomplished by cooperative action with excitatory amino acid transporters, which import glutamate for re-export by system X_c^- as driving force for cystine import. Additionally, the glutamate resistant cells showed higher expression of catalase, ferritin, and aldehyde dehydrogenase-3, all known to be involved in antioxidant defense. Furthermore, we could identify the upregulated homeobox transcription factor Pem as protective.

2. Ischemic preconditioning

Julius Steinbeck, Susanne Thomsen, Frank Leypoldt, Benedikt Westphalen

Short episodes of ischemia can protect neuronal cells and tissue against a subsequent lethal ischemia. This so-called ischemic preconditioning depends on ischemia-induced gene transcription. Subtractive suppression hybridization was employed to identify transcripts upregulated in primary neurons, protected by ischemic preconditioning.

Transcriptional analysis. One upregulated transcript corresponded to the antiapoptotic gene Bax inhibitor-1, known to interact with Bcl-2, a key mediator of ischemic preconditioning. The functional significance of this upregulation could be demonstrated by tetracycline-inducible overexpression in HEK293 cells, which mediated protection against oxygen-glucose deprivation. We also could show, that Bax inhibitor-1 overexpression has profound effects on endoplasmic reticulum calcium signaling, indistinguishable from those induced by Bcl-2. Thus, modifying calcium homeostasis might represent the pathway through which both proteins together prevent neuronal death. Another upregulated sequence tag could be identified as the

previously unknown 3' untranslated region of the non-catalytic growth factor receptor TrkB.T1.

Support

The work was supported by the DFG (now transferred to W. Hampe as principal investigator of the SorLA/LR11 project).

Support of guest group Methner

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Publications

(1) Hentschke, M., Süsens, U., Borgmeyer, U. (2002). Domains of ERRγ that mediate homodimerization and interaction with factors stimulating DNA binding. Eur. J. Biochem. 269, 4086-4097.

(2) Hentschke, M., Süsens, U., Borgmeyer, U. (2002). PGC-1 and PERC, coactivators of the estrogen receptor-related receptor γ. Biochem. Biophys. Res. Commun. 299, 872-879.

(3) Hoffmeister-Ullerich, S., Herrmann, D., Kielholz, J., Schweizer, M., Schaller, H. C. (2002). Isolation of a putative peroxidase, a target for factors controlling foot-formation in the coelenterate hydra. Eur. J. Biochem. 269, 4597-4606.

(4) Lintzel, J., Franke, I., Riedel, I. B., Schaller, H. C., Hampe W. (2002). Characterization of the VPS10 domain of SorLA/LR11 as binding site for the neuropeptide HA. Biol. Chem. 383, 1727-1733.

(5) Riedel, I. B., Hermans-Borgmeyer, I., Hübner, C. A. (2002). SorLA, a member of the LDL receptor family, is expressed in the collecting duct of the murine kidney. Histochem. Cell Biol. 118, 183-191.

(6) Venero, C., Tilling, T., Hermans-Borgmeyer, I., Schmidt, R., Schachner, M., Sandi, C. (2002). Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neuroscience 115, 1211-1219.

(7) Wittenberger, T., Hellebrand, S., Munck, A., Kreienkamp, H.-J., Schaller, H. C., Hampe, W. (2002). GPR99, a new G protein-coupled receptor with homology to a new subgroup of nucleotide receptors. BMC Genomics 3, 17.

(8) Barreto, G., Borgmeyer, U., Dreyer, C. (2003). The germ cell nuclear factor is required for retinoic acid signaling during Xenopus development. Mech. Dev. 120, 415-428.

(9) Boels, K., Schaller, H. C. (2003). Identification and characterisation of GPR100 as a novel human G-protein-coupled bradykinin receptor. Br. J. Pharmacol. 140, 932-938.

(10) Gutekunst, C.-A., Torre, E. R., Sheng, Z., Yi, H., Coleman, S. H., Riedel, I. B., Bujo, H. (2003). Stigmoid bodies contain type I receptor proteins SorLA/LR11 and

Sortilin: New perspectives on their function. J. Histochem. Cytochem. 51, 841-852.

(11) Hentschke, M. and Borgmeyer, U. (2003). Identification of PNRC2 and TLE1 as activation function-1 cofactors of the orphan nuclear receptor ERRγ. Biochem. Biophys. Res. Commun. 312, 975-982.

(12) Hentschke, M., Schulze, C., Süsens U., and Borgmeyer, U. (2003). Characterization of calmodulin binding to the orphan nuclear receptor ERRγ. Biol. Chem. 384, 473-482.

(13) Ignatov, A., Lintzel, J., Kreienkamp, H.-J., Schaller, H. C. (2003). Sphingosine-1-phosphate is a high-affinity ligand for the G protein-coupled receptor GPR6 from mouse and induces intracellular Ca^{2+} release by activating the sphingosine-kinase pathway. Biochem. Biophys. Res. Commun. 311, 329-36.

(14) Ignatov, A., Lintzel, J., Hermans-Borgmeyer, I., Kreienkamp, H.-J., Joost, P., Thomsen, S., Methner, A., Schaller, H. C. (2003). Role of the G-Protein-coupled receptor GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J. Neurosci. 23, 907-914.

(15) Putthoff, P., Akyuz, N., Kutsche, M., Zardi, L., Borgmeyer, U., Schachner, M. (2003). Structure of the murine tenascin-R gene and functional characterisation of the promoter. Biochem. Biophys. Res. Commun. 308, 940-949.

(16) Urny, J., Hermans-Borgmeyer, I., Gercken, G., Schaller, H.C. (2003). Expression of the presenilin-like signal peptide peptidase (SPP) in mouse adult brain and during development. Gene Expr. Patterns 3, 685-691.

(17) Scherzer, C. R., Offe, K., Gearing, M., Ress, H. D., Fang, G., Heilman, C. J., Schaller, H. C., Levey, A. I., Lah, J. J. (2003). ApoE receptor LR11 in Alzheimer's disease: Gene profiling of lymphoblasts mirrors changes in the brain. Arch. Neurol., in press.

(18) Hermey, G., Plath, N., Hübner, C. A., Kuhl, D., Schaller, H.C., Hermans-Borgmeyer, I. (2004). The three sorCS genes are differentially expressed and regulated by synaptic activity. J. Neurochem. 88, 1470-1476.

(19) Ignatov, A., Hermans-Borgmeyer, I., Schaller, H. C. (2004). Cloning and characterization of a novel G-protein-coupled receptor with homology to galanin receptors. Neuropharmacology, in press.

Publications of guest group Methner

(1) Leypoldt, F., Flajolet, M., Methner, A. (2002). Neuronal differentiation of cultured human NTERA-2cl.D1 cells leads to increased expression of synapsins. Neurosci. Lett. 324, 37-40.

(2) Joost, P., Methner, A. (2002). Phylogenetic analysis of 277 human G-protein-coupled receptors as a tool for the prediction of orphan receptor ligands. Genome Biol. 3, RESEARCH0063.

(3) Lewerenz, J., Gocht, A., Hoeger, P. H., von den Driesch, P., Eckert, B., Lamszus, K., Stuerenburg, H-J., Methner, A. (2003). Multiple vascular abnormalities and a paradoxical combination of vitamin B12 deficiency and thrombocytosis in a case with POEMS syndrome. J. Neurol. 250, 1488-91.

(4) Lewerenz, J., Letz, J., Methner, A. (2003). Activation of stimulatory heterotrimeric G proteins increases glutathione and protects neuronal cells against oxidative stress. J. Neurochem. 87, 522-31.

(5) Lewerenz, J., Leypoldt, F., Methner, A. (2003). Degenerate suppression PCR identifies the beta2-adrenergic receptor as upregulated by neuronal differentiation. Gene Expr. 11, 105-16.

(6) Ignatov, A., Lintzel, J., Hermans-Borgmeyer, I., Kreienkamp, H.-J., Joost, P., Thomsen, S., Methner, A., Schaller, H. C. (2003). Role of the G-protein-coupled receptor GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J. Neurosci. 23, 907-14.

Dissertations

Rezgaoui, Meriem (2002). Identifizierung neuer Vps10p-Rezeptoren und vergleichende Analyse ihres Expressionsmusters. Fachbereich Biologie, Universität Hamburg.

Urny, Jens (2003). Identifizierung und Charakterisierung der Signalpeptid-Peptidase SPPα und der Spleißvariante SPPβ. Fachbereich Chemie, Universität Hamburg.

Bader, Christian-Olaf (2003). Etablierung einer quantitativen kompetitiven RT-PCR zur Bestimmung der SorLA-mRNS-Expression. Fachbereich Medizin, Universität Hamburg.

Hentschke, Moritz (2003). DNS-Bindung und Regulation des nukleären Waisenrezeptors Estrogen receptor-related receptor γ (ERRγ). Fachbereich Medizin, Universität Hamburg.

Ignatov, Atanas (2003). Identification of endogenous ligands for the closely related orphan G-protein-coupled receptors GPR6 and GPR12. Fachbereich Medizin, Universität Hamburg.

Habilitation

Hoffmeister-Ullerich, Sabine (2002). Peptide und ihre Funktion bei der Kontrolle von Wachstums- und Differenzierungsprozessen, analysiert im Modellsystem Hydra. Im Fach Zoologie mit Schwerpunkt Entwicklungsbiologie, Fachbereich Biologie, Universität Hamburg.

Dissertations of guest group Methner

Lewerenz, Jan (2003). Molecular mechanisms of protection against oxidative glutamate toxicity in the neuronal cell line HT22. Fachbereich Medizin, Universität Hamburg.

Leypoldt, Frank (2004). Identifikation von transkriptionell hochregulierten Genen bei der Differenzierung von humanen, neuronalen Vorläuferzellen Ntera-2cl.D1. Fachbereich Medizin, Universität Hamburg.

Awards of guest group Methner

Best poster prize Deutsche Gesellschaft für Neurologie, annual meeting 2001, to Axel Methner.

Best poster prize Deutsche Gesellschaft für Neurologie, annual meeting 2002, to Jan Lewerenz.

Arthur Kling, MD Award to Axel Methner, 2003.

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Institut für Molekulare Neuropathobiologie

Thomas J. Jentsch

Our research is concerned with ion transport processes, in particular with the CLC chloride channel family, as well as with KCNQ K^+ channels and KCC K-Cl cotransporters. We study the structure and function of these ion transport proteins and are very interested in their role for the cell and the organism as a whole. We have elucidated several inherited human diseases that are caused by mutations in Cl^- or K^+ channels, and have generated and analysed a large number of knock-out (KO) mouse models. These animal models led our research into different organ systems, including the brain, eye, inner ear, intestine, kidney, and bone. For instance, we are currently working on four different genes, mutations in which cause deafness in humans or mice.

Most of our research focuses on the family of CLC Cl^- channels, which we discovered at the ZMNH back in 1990. In mammals, there are nine different CLC genes that can be grouped into three branches by homology. The first branch (ClC-1, -2, -Ka and Kb) represents channels which exert their function in the plasma membrane. The other two branches, ClC-3/4/5 and ClC-6/7, respectively, represent channels that are localized predominantly, but not exclusively, in vesicles of the endocytotic pathway. They are thought to be necessary for an efficient acidification of these organelles. So far, only one β-subunit, barttin, is known for CLC channels. It associates exclusively with ClC-Ka and ClC-Kb and is crucial for their transport to the plasma membrane.

The crystallization of a bacterial CLC protein by Dutzler, MacKinnon and colleagues greatly facilitates structure-function studies. We have exploited the crystal structure in mapping an inhibitor binding site in the skeletal muscle chloride channel ClC-1 (5). After our identification of barttin as the first β-subunit of CLC channels, we are continuing our search for other interaction partners. The main focus of our work in this area, however, is the physiology and pathophysiology of CLC channels which we address with mouse models and the elucidation of human diseases. These pathologies (myotonia, renal salt loss, proteinuria, deafness, osteopetrosis, blindness, CNS degeneration…) underscore the importance of Cl^- channels and reveal their diverse and specific functions.

Several years ago, we began studying KCC K-Cl cotransporters because they are likely to influence intracellular Cl^- in neurons, a role also attributed to the ubiquitously expressed Cl^- channel ClC-2. Indeed, our KO mouse models have shown that KCC2 is the main player in determining Cl^- in neurons, with a minor role played by KCC3 (6). The upregulation of KCC2 during development (7) is essential for the 'switch' of the response to GABA from excitatory to inhibitory.

Our recent KOs of KCC3 (6) and KCC4 (1), however, have also revealed or confirmed other functions of KCl cotransport. We have e.g. shown that both cotransporters play a role in cell volume regulation. Interestingly, both transporters are expressed in the K^+ recycling pathway of the inner ear and the KO of either isoform leads to deafness, albeit with very different time courses.

Finally, we continue our studies on KCNQ channels, which we have previously shown to be involved in epilepsy and

deafness. In addition to structure-function investigations, we now mainly focus on mouse models and diseases.

1. Structure and function of CLC Cl^- channels

In spite of the physiological and medical importance of CLC Cl^- channels, their pharmacology is poorly developed. The skeletal muscle Cl^- channel ClC-1 is inhibited by 0.1 mM anthracene-carboxylic acid (9-AC), while ClC-0 and ClC-2 are nearly unaffected at this concentration. We used chimeras between ClC-1 and ClC-2 to identify a serine residue that is critical for inhibitor sensitivity (5). The crystal structures of bacterial CLC proteins that were subsequently published by Dutzler et al. revealed that this residue is close to the central Cl^- binding site and should be accessible from the cytoplasm, consistent with the sidedness of the effect of 9-AC. We then used the crystal structure of the bacterial protein and mutated many residues predicted to be close to that serine to delineate the 9-AC binding site. This work defined a partially hydrophobic inhibitor binding pocket on the cytoplasmic side of the narrowest part of the pore (Fig. 1). Our work demonstrates that the crystal structures of bacterial CLC proteins, which are now known to be $2Cl^-/H^+$ exchangers rather than Cl^- channels, can be extrapolated with high fidelity to mammalian Cl^- channels (5).

The carboxy-termini of each subunit of eukaryotic CLC channels contain two CBS (cystathionine-β-synthase) domains, which are not present in the bacterial CLC proteins that were crystallized and the function of which is unclear. These domains are found in many different proteins. In previous studies we had shown that mutations in the CBS domains of the single yeast CLC led to its mislocalization. Channels deleted for a carboxy-terminal stretch containing CBS2 did not yield currents, but could be rescued by the co-expression of that fragment. Using primarily ClC-1, we have now shown that the CBS domains interact with each other and are necessary for the transport of the channel to the surface (8). The CBS domains are largely interchangeable. For instance, ClC-1 is still functional when its CBS domain is replaced by that of the enzyme inosine monophosphate

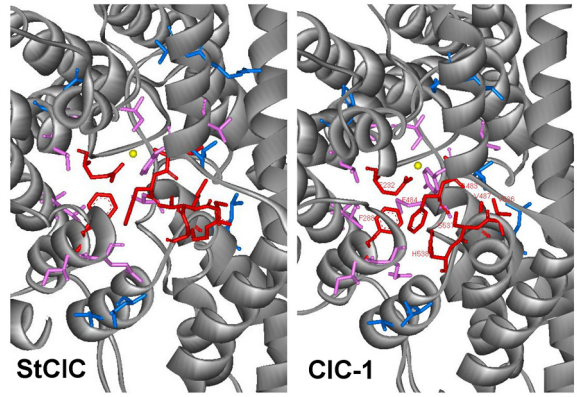

Figure 1: Inhibitor binding site in CLC proteins. The left picture shows a part of the crystal of the bacterial protein StClC as described by Dutzler et al., and the right picture the deduced structure of the corresponding part of the skeletal muscle Cl^- channel ClC-1. The Cl^- ion as apparent in the crystal of the bacterial protein StClC is shown as a ball. The side chains of the amino acids whose mutations strongly influence the binding of the inhibitor 9-AC are shown in red. Side chains of amino acids having no or minor effect on binding are shown in blue or pink, respectively. These experiments define an inhibitor binding pocket and demonstrate the high degree of conservation between these proteins. From: Estévez R., Schroeder B.C., Accardi A., Jentsch T.J., Pusch M. (2003). Conservation of chloride channel structure revealed by an inhibitor binding site in ClC-1. *Neuron* 38, 47-59.

dehydrogenase. Interestingly, many mutations (including point mutations) in CBS domains affect the gating of the channel (8), compatible with a regulatory role, and mutations in CBS domains were found in patients with CLC channelopathies.

2. CLC mouse models

In order to understand the diverse physiological functions of CLC channels and to generate animal models for human disease, we have knocked out the genes encoding ClC-2, ClC-3, … to ClC-7. During the past reporting period, we have published first papers on ClC-2 (*EMBO J.* 20, 1289 (2001)), ClC-3 (*Neuron* 29, 185 (2001)), ClC-5 (*Nature* 408: 369 (2000)), and ClC-7 (*Cell* 104, 205 (2001)) knock-out mouse models. Our analysis of ClC-4 and ClC-6 KO mice is still ongoing, as these do not have an overt phenotype. In addition, we are continuing the analysis of our published mouse models, which includes expression profiling using the Affymetrix technology.

In addition to the plasma membrane channels, we investigate the vesicular Cl^- channels ClC-3 to ClC-7. They reside in cytoplasmic vesicles of the endocytotic-lysosomal pathway and also on synaptic vesicles (ClC-3), in which their expression overlaps to some extent. They are thought to facilitate the acidification of these vesicles by providing an electric shunt for the proton pump, as we have shown directly for ClC-5 in kidney endosomes (14) and for ClC-3 in synaptic vesicles. The importance of this acidification is best understood in the case of ClC-5, where the knock-out leads to a defect in endocytosis, and in the case of ClC-7, where the defective acidification of the resorption lacuna of osteoclasts results in osteopetrosis in mice and man carrying non-functional ClC-7. We have found that the ClC-7 KO has a severe CNS degeneration in addition to osteopetrosis. Consistent with the lysosomal localization of ClC-7, it displays the typical hallmarks of a lysosomal storage disease. Using the TRAP-promoter to express the ClC-7 cDNA specifically in osteoclasts, we rescue the bone phenotype of ClC-7 KO mice.

To eliminate possible redundancies between these vesicular channels, we are generating and analyzing double-KO animals which often display more severe phenotypes.

3. ClC-2 and CFTR: a modulatory role of ClC-2 in cystic fibrosis?

Cystic fibrosis (CF) is a severe inherited disease which affects mainly the lung, pancreas, and liver. It is caused by mutations in CFTR, a cAMP-activated Cl^- channel that belongs to the family of ABC-transporters. CFTR has an important function in transepithelial transport across several epithelia, where CFTR mediates Cl^- transport across the apical membrane.

It has been speculated for a long time that Cl^- channels that are present in the same apical membranes as CFTR might modulate the CF phenotype and that their pharmacological activation may be useful in treating CF. As ClC-2 is ubiquitously expressed and has been described by some groups to reside in apical membranes of epithelia, a double KO of CFTR and ClC-2 (Bösl et al., *EMBO J.* 20: 1289 (2001)) might be expected to lead to a more severe defect in transepithelial transport than the single CFTR KO.

Contrary to these expectations, however, double KOs generated in our laboratory did not display a more severe CF phenotype (9). In particular, the lung and the pancreas, which (in contrast to humans) are not affected in CFTR mouse models, did not show pathological alterations in the

double KO either. Surprisingly, mice homozygous for the common ΔF508 CFTR mutation and additionally deleted for ClC-2 (Bösl et al., *EMBO J.* 20: 1289 (2001)) even survived better than ΔF508 mice. Ussing chamber experiments revealed that cAMP-stimulated Cl^- secretion is enhanced rather than decreased in *Clcn2*$^{-/-}$ mice as compared to WT. This increase in currents is compatible with a basolateral rather than apical localization of ClC-2 and might explain the better survival of *Cftr*$^{\Delta F508/\Delta F508}$/*Clcn2*$^{-/-}$ mice (9). Thus, activation of ClC-2 will not be useful in treating CF.

4. Accessory subunits of CLC channels

We have shown in 2001 (Estévez et al. *Nature* 416, 874) that Barttin, a membrane protein with two transmembrane domains that is mutated in Bartter syndrome type IV (presenting with renal salt loss and deafness), acts as an accessory β-subunit of ClC-Ka and ClC-Kb. It is colocalized with these Cl^- channel α-subunits in renal epithelia and in the stria vascularis of the cochlea and is necessary for the transport of the channel complex to the plasma membrane. ClC-K/barttin channels provide the basolateral exit pathway for Cl^- reabsorption in the nephron and for Cl^- recycling in the stria vascularis (Fig. 2). We are generating Barttin mouse models to get a better understanding of its function in both the kidney and the inner ear. Further, we are looking for interaction partners of this and other CLC Cl^- channels.

5. Functions of KCC K-Cl cotransporters revealed by KO mouse models

KCC proteins co-transport K^+ and Cl^- ions in an electroneutral manner. There are four different isoforms encoded by different genes (*Kcc1* through *Kcc4*).

KCC proteins play a role in transepithelial transport, cell volume regulation, and in the determination of intracellular Cl^- concentration in neurons. The latter function is crucial for synaptic inhibition, as $[Cl^-]_i$ determines the electrical response of $GABA_A$- and glycine-receptors. The generation and analysis of KCC KO mice allowed us to assess the relevance

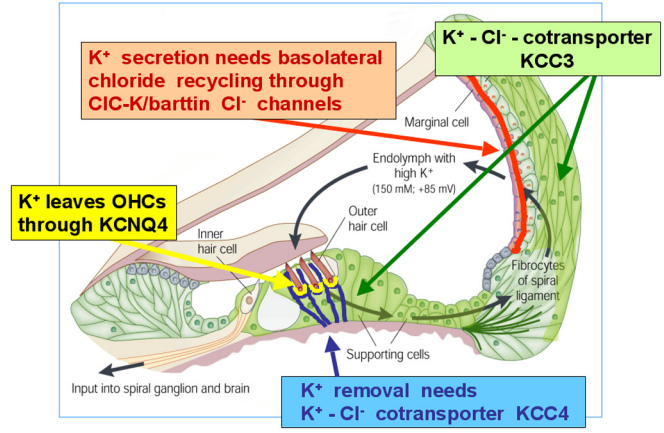

Figure 2.: Model of K^+ recycling in the inner ear. We are working on four transport proteins that may have a role in K^+ recycling and that lead to deafness in man or mice when mutated: Barttin, together with the ion-conducting subunits ClC-Ka and ClC-Kb, is necessary for K^+ secretion of the stria vascularis because it is needed for the basolateral recycling of Cl^-. The resulting high K^+ concentration of the endolymph is necessary for the hearing process as it drives K^+ through apical mechanosensitive channels into sensory hair cells. K^+ leaves outer hair cells through KCNQ4 K^+ channels, mutations in which lead to human deafness of the DFNA2 type. K^+ is then taken up by supporting Deiter's cells, probably through a combination of the K-Cl cotransporters KCC3 and KCC4. KO of either transporter gene leads to deafness in mice. KCC3 is also expressed in other cells of the epithelial and fibrocyte gap junction system which is thought to recycle K^+ back to the stria vascularis for another round of secretion.

of K-Cl cotransport for these functions and to discover new, unexpected roles of KCC proteins.

KCC2 is a neuronal isoform that is the main player in determining $[Cl^-]_i$, as shown by the constitutive KO we have previously generated and analysed (Hübner et al., *Neuron* 30, 515 (2001)). Its transcription is upregulated after birth and parallels the development of an inhibitory response to GABA (7). The initial excitatory effect of GABA, which is due to a high value of $[Cl^-]_i$, is thought to be important for the development of the CNS. We are currently working on several KCC2 mouse models to further understand the role of $[Cl^-]_i$ in brain function.

While the constitutive KO of KCC2 led to postnatal lethality, KCC3 KO mice (6) are being severely affected but survive. They display a severe degeneration of the PNS and CNS, reduced seizure threshold and EEG abnormalities, high blood pressure, and a slowly progressive hearing loss. KCC3 mutations also underlie a human disease, Anderman syndrome, as described by Rouleau and colleagues (*Nat. Genet*. 32, 384 (2002)). The hallmarks of Anderman syndrome are peripheral neurodegeneration and a variable agenesis of the corpus callosum.

KCC3 is broadly expressed in neuronal and non-neuronal cells. In the inner ear, KCC3 is expressed in supporting cells of sensory hair cells and in cells of the epithelial and fibrocyte gap junction systems, with the exception of type II fibrocytes (Fig. 2). It may play a role in potassium recycling. The slowly progressive hearing loss in the KO mutant is paralleled by a degeneration of the organ of Corti (6). It may be due to defective K^+ recycling, or to defective cell volume regulation.

On the cellular level, we have shown that KCC3 is important for the regulatory volume decrease in proximal tubular cells and cultured neurons, which may contribute to the neurodegeneration. The increased electrical excitability of the CNS may be caused by an increase in $[Cl^-]_i$, which we have found by using perforated patch measurements of brain slices. Compared to KCC2, however, the effect of KCC3 on $[Cl^-]_i$ was minor (6).

The KO of KCC4, an isoform almost exclusively expressed in non-neuronal cells, led to mice that were viable and lacked an immediately visible phenotype (1). However, these mice rapidly developed deafness that was paralleled by a degeneration of hair cells and the organ of Corti which occured within a few weeks after the onset of hearing. In the mature inner ear, KCC4 is exclusively expressed in the supporting cells of sensory hair cells (1) (Fig. 2). We hypothesize that it serves to remove K^+ from the outer hair cells and to funnel it into the gap junction recycling pathway.

KCC4 KO mice additionally present with renal tubular acidosis (1). In the kidney, KCC4 is expressed in the basolateral membranes of proximal tubular cells and in intercalated cells of the collecting duct. α-intercalated cells secrete acid into the lumen, using an apical proton pump and a basolateral anion exchanger. Cl^- ions exchanged for bicarbonate have to be recycled over the basolateral membrane. Our data (1) suggest that KCC4 plays a prominent role in this recycling step.

6. KCNQ K^+ channels: structure, function, and role in disease

We have previously cloned KCNQ2, -3, -4, and -5 K^+ channels and have shown that KCNQ2 and KCNQ3 are involved in a rare form of human neonatal epilepsy, while KCNQ4 is mutated in DFNA2, an autosomal dominant, slowly progressive hearing loss in humans.

KCNQ3 can form heteromers with KCNQ2, KCNQ4, and KCNQ5, but not with KCNQ1. Using a chimeric strategy, we identified a carboxy-terminal domain (SID, 'subunit-interaction-domain') in KCNQ channels that specifies the assembly preference (4). For instance, transplantation of this domain from KCNQ3 into KCNQ1 enables assembly with KCNQ2, 3, 4, and 5. In the field of channelopathies, we continue our collaboration with Ortrud Steinlein and analyse the biophysical effects of KCNQ2 mutations she identifies in patients with benign familial neonatal convulsions (BFNC) (3).

The main focus of our work on KCNQ channels is now on mouse models, in particular for deafness that is caused by mutations in the KCNQ4 K^+ channel. In the mature inner ear, KCNQ4 is expressed in the basal pole of outer hair cells, where it may mediate K^+ efflux (Fig. 2). The slow progression of DFNA2 deafness suggests the channel itself is not necessary for the hearing process, but that inactivating mutations lead to a slow degeneration of sensory hair cells.

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Publications

(1) Boettger T., Hübner C.A., Maier H., Rust M., Beck F.X., Jentsch T.J. (2002). Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter KCC4. *Nature* 416, 874-878.

(2) Riedel I.B., Hermans-Borgmeyer I., Hübner C.A. (2002). SorLA, a member of the LDL receptor family, is expressed in the collecting duct of the murine kidney. *Histochem. Cell Biol.* 118, 183-191.

(3) Dedek K., Fusco L., Teloy N., Steinlein O.K. (2003) Neonatal convulsions and epileptic encephalopathy in an Italian family with a missense mutation in the fifth transmembrane region of KCNQ2. *Epilepsy Res.* 54, 21-27.

(4) Schwake M., Jentsch T.J., Friedrich T. (2003). A carboxy-terminal domain determines the subunit specificity of KCNQ K^+ channel assembly. *EMBO Reports* 4, 76-81.

(5) Estévez R., Schroeder B.C., Accardi A., Jentsch T.J., Pusch M. (2003). Conservation of chloride channel structure revealed by an inhibitor binding site in ClC-1. *Neuron* 38, 47-59.

(6) Boettger T., Rust M.B., Maier H., Seidenbecher T., Schweizer M., Keating D., Faulhaber J., Ehmke H., Pfeffer C., Scheel O., Lemcke B., Host J., Leuwer R., Pape H.C., Völkl H., Hübner C.A., Jentsch T.J. (2003). Loss of K-Cl cotransporter KCC3 causes deafness, neurodegeneration and reduced seizure threshold. *EMBO J.* 22, 5422-5434.

(7) Stein V., Hermans-Borgmeyer I., Jentsch T.J., Hübner C.A. (2004). Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride *J. Comp. Neurol.* 468, 57-64.

(8) Estévez R., Pusch M., Ferrer-Costa C., Orozco M., Jentsch T.J. (2004). Functional and structural conservation of CBS domains from CLC channels. *J. Physiol.*, in press.

(9) Zdebik A.A., Cuffe J., Bertog M., Korbmacher C., Jentsch T.J. (2004). Additional disruption of the ClC-2 Cl^- channel does not exacerbate the cystic fibrosis phenotype of CFTR mouse models. *J. Biol. Chem.*, in press.

Review Articles

(10) Jentsch, T.J. (2002). Chloride channels are different (News & Views). *Nature* 415, 276-277.

(11) Jentsch T.J., Stein V., Weinreich F., Zdebik A.A. (2002). Molecular structure and physiological function of chloride channels. *Physiol. Rev.* 82, 503-568.

(12) Estévez R., Jentsch T.J. (2002). CLC chloride channels: Correlating structure with function. *Curr. Opin. Struct. Biol.* 12, 531-539.

(13) Hübner C.A., Jentsch T.J. (2002). Ion channel diseases. *Hum. Mol. Genet.* 11, 2435-2445.

(14) Günther W., Piwon N., Jentsch T.J. (2003). The ClC-5 chloride channel knock-out mouse - an animal model for Dent's disease. *Pflügers Arch. Eur. J. Physiol.,* 445, 456-462.

Theses

Diploma

Lange, Philipp (2004). Universität Hamburg

Meyer, Sebastian (2004). Universität Hamburg

Stephan, Alexander (2003). Universität Hamburg

Dissertations

Dedek, Karin (2003). Universität Oldenburg

Kharkovets, Tatjana (2003). Universität Hamburg

Schaffer, Sven (2002). Universität Hamburg

Awards

Berliner Professor, Yale University, 2003

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Homer W. Smith Award (American Society of Nephrology), 2004

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A fundamental property of neurons is the generation and propagation of electrical signals. They are generated by flow of ions across the neural membrane upon excitation. Since the classical work of Hodgkin and Huxley it is known how different voltage-dependent conductances contribute to the propagated action potential. The molecular approach to neurobiology revealed that different membrane proteins forming voltage-gated ion channels selective for potassium, sodium, or other ions are the basic units of biological excitability and that the concerted opening and closing of these channels determines the waveform of the generated action potential. Many genes encode potassium channels yielding a potentially bewildering number of potassium channels with diverse properties. But only a few of the potassium channels have been associated with heritable diseases. Furthermore, a common hallmark of potassium channel-related diseases is their episodic nature. Thus, the link between the expression of a dysfunctional potassium channel and the pathophysiological phenotype is often not obvious. In recent years, mutations in a few potassium-channel genes were discovered which were associated with long and short forms of the arrhythmogenic cardiac QT syndrome and, respectively, with a benign form of juvenile epilepsy. Accordingly, we have extended our work on the structure, function and physiology of potassium channels to these channels, in particular the cardiac I_{Ks} and I_{Kr} channels and neuronal M-channels to specifically address the molecular bases of episodic nature of the human diseases that are correlated with the activity of these channels.

1. Cardiac ion channel activity and arrhythmia

Dirk Isbrandt, Axel Neu, Patrick Friederich, Chi-un Choe, Anna Solth, Kathrin Sauter, Stefan Schillemeit

Dynamic regulation of contraction rate and force of the heart is an essential property of the cardiovascular system and crucial for the adaptation of blood flow in response to environmental factors. It is, in particular, the stimulation of the sympathetic nervous system (SNS) that in response to exercise or emotional stress can lead to an immediate and dramatic increase in heart rate, which is paralleled by a concomitant decrease in action potential duration of cardiac myocytes and a shortening of the QT interval of the surface electrocardiogram. To a large extent these effects are mediated by activation of β-adrenergic receptors that regulate the activity of specific cardiac ion channels via increases in intracellular cAMP concentrations. Mutations in cardiac ion channel genes that lead to altered channel function or channel regulation may thus lead to syndromes associated with cardiac rhythm disturbances.

Our past and present research focuses on the molecular basis of inherited cardiac disorders such as the long QT syndrome (LQTS) and sinus node dysfunction (SND). By characterizing ion channel dysfunction resulting from gene mutations we aim to get insight into the pathophysiological bases of these diseases.

Long QT syndrome

The long QT syndrome (LQTS) is a cardiac disorder that increases the risk of sudden death. The disease is

characterized by a prolongation of the QT interval on the electrocardiogram. Patients suffer from syncopal episodes due to ventricular arrhythmias like *Torsade de pointes* and a high risk of sudden cardiac death. In collaboration with Dr. E. Schulze-Bahr (Department of Cardiology, University Hospital Münster) we identified and functionally characterized novel LQTS-associated mutations in the potassium channel a subunits *HERG* and *KCNQ1* and in their auxiliary subunits *KCNE1* and *KCNE2*. The LQTS-associated mutations caused heterogeneous *in vitro* phenotypes including altered intracellular trafficking, reduced subunit stability, and altered biophysical properties that may explain the clinical phenotype. Interestingly, some C-terminal HERG mutations showed under standard conditions only minute changes in their properties. However, when b-adrenergic, cAMP-dependent channel regulation was investigated, mutant HERG channel activity did not increase like in wild-type channels. These findings demonstrate the physiological importance of coupling b-adrenergic stimulation and HERG channel activity, which is key to SNS control of cardiac electric activity.

Sinus node dysfunction

SND is the major cause necessitating pacemaker implantation and accounts for approximately half of all patients requiring a pacemaker. The disease commonly occurs in adults with acquired heart disease, during antiarrhythmic therapy, or after surgically corrected, congenital heart disease. In a significant portion of patients, however, SND appears in the absence of identifiable cardiac abnormalities or other associated conditions ('idiopathic' SND). The cardiac pacemaker current I_f is a major determinant of diastolic depolarization in sinus nodal cells and has a key role in heart beat generation. We hypothesized that some forms of 'idiopathic' sinus node dysfunction (SND) may be related to inherited dysfunctions of cardiac pacemaker (HCN) channels. In a candidate gene approach we detected a heterozygous mutation in the human *HCN4* gene of a patient with idiopathic SND. The mutation resulted in a mutant HCN4 protein with a truncated C-terminus that lacked the cyclic nucleotide-binding domain. Immunocytochemical experiments indicated normal intracellular trafficking and membrane integration of mutant HCN4 subunits. However, patch-clamp experiments showed that the mutant HCN4 channels mediated I_f -like currents that were insensitive to increased cellular cAMP levels. Furthermore, coexpression experiments revealed a dominant-negative effect of mutant HCN4 subunits on wild-type subunits. Together, the clinical, genetic, and *in vitro* data provided a likely explanation for the patient's sinus bradycardia and the chronotropic incompetence. Our work was the first to describe a naturally occurring human mutation in channels of the HCN family and for the first time linked an ion channel mutation to SND.

2. Structure-based mutational analysis of cardiac potassium channels.

Robert Bähring, Roman Born, Lijuan Ma, Nicole Schmitt**

KCNQ1 is a member of the KCNQ family of K^+ channels, which encodes voltage-gated K^+ (Kv) channels with important physiological functions. Coassembly of KCNQ1 channels with the β subunit KCNE1 generates a delayed-rectifier K^+ current underlying I_{Ks}, a key component in controlling the duration of the action potential in the human heart.

KCNQ1 channels have several remarkable biophysical properties which are altered upon association with KCNE1 subunits. These include a slowing of activation, a suppression of inactivation and an increase in apparent single-channel conductance. In addition, Rb^+ currents conducted by homomeric KCNQ1 channels are about threefold larger than K^+ currents, whereas heteromeric KCNQ1-KCNE1 have smaller inward Rb^+ currents compared to K^+ currents. The location of MinK relative to KCNQ1 in KCNQ1/KCNE1 channels is a subject of debate. It has been proposed that KCNE1 residues gain exposure to the outer pore vestibule, travel close to the ion conduction pathway near the selectivity filter, and influence the structure of the inner pore vestibule. We used tryptophan-scanning mutagenesis comprising amino-acid residues of outer- (S5/TM1), pore- (P) and inner- (S6/TM2) helices as a tool to explore protein-protein and protein-lipid interfaces on the outer transmembrane shell of the pore domain of human KCNQ1 and KCNQ1/KCNE1 channels. We mapped the results of our tryptophan-scanning mutagenesis onto the KcsA crystal structure to predict interacting sites between KCNQ1 pore domain, voltage sensor and KCNE1, and between KCNQ1 pore domain and lipid. In contrast to *Shaker* channels, the emerging pattern is in very good agreement with the proposed structural model for the bacterial KvAP channel. In this structure, the upper half of the transmembrane shell of the KvAP pore domain mostly interfaces with lipid molecules and the lower half is mostly engaged with the voltage-sensing machinery. Our results suggests that major features of the KvAP structure have been conserved in human KCNQ1 channels.

3. Functional analysis of neuronal KCNQ/M-channels using transgenic mouse models

Dirk Isbrandt, Quyen Le, Howard Christian Peters, Axel Neu

Heteromeric voltage-gated potassium channel subunits of the KCNQ family are the molecular correlates of the native M-current that regulates subthreshold electrical excitability of many neurons. The M-current is thought to play a crucial role in the generation of medium afterhypolarisations (mAHPs) and early spike frequency adaptation in hippocampal pyramidal neurons. Mutations in either KCNQ2 or KCNQ3 co-segregate with benign familial neonatal convulsions (BFNC), a neonatal form of epilepsy.

We generated a functional knockout of the native M-current in neurons through inducible expression of a dominant-negative hKCNQ2 subunit under the control of the human prion protein promotor. Electrophysiological studies with acute brain slices revealed that mutant hippocampal CA1 neurons have normal membrane resting potentials, but, when stimulated, exhibited abnormally high action potential frequencies followed by markedly reduced afterhyperpolarizations. Most remarkably, the typical subthreshold resonance of CA1 neurons in the theta frequency range is lost. Mutant female mice with a mosaic expression pattern showed hippocampus-dependent memory deficits. Surprisingly, we found abnormal dispersion of hippocampal CA1 neurons in male mutant mice with a widespread expression of the dominant-negative KCNQ2 subunits. Mutant male mice were conspicuously hyperactive and suffered spontaneous seizures. Our findings suggest

that hyperexcitability and seizure susceptibility are associated with altered M-channel activity.

4. BK-Channel activity, arterial tone and hypertension.

Saskia Plüger, Ralph Waldschütz*, in collaboration with Jörg Faulhaber and Heimo Ehmke*

Large-conductance potassium (BK) channels in vascular smooth muscle cells (VSMCs) sense both changes in membrane potential and in intracellular Ca^{2+} concentration. BK channels may serve as negative feedback regulators of vascular tone by linking membrane depolarization and local increases in intracellular Ca^{2+} concentration (Ca^{2+} sparks) to repolarizing spontaneous transient outward K+ currents (STOCs). BK channels are composed of channel-forming BK and auxiliary BKβ1 subunits, which confer to BK channels an increased sensitivity for changes in membrane potential and Ca^{2+}. To assess *in vivo* functions of BK-channels in smooth muscle, mice with a disrupted BKβ1 gene were generated. Our results indicate that BKβ1 -/- mice have an abnormal Ca^{2+} spark/STOC coupling that is shifted to more depolarized potentials. Thoracic aortic rings from BKβ1 -/- mice responded to agonist and elevated KCl with an increased contractility. BKβ1 -/- mice had higher systemic blood pressure than BKβ1 +/+ mice but responded normally to α1-adrenergic vasoconstriction and nitric oxide–mediated vasodilation. Furthermore, elevated blood pressure in BKβ1 -/- mice is associated with hyperaldosteronism and altered renin/prorenin concentrations in the blood. Based on extensive pharmacological and physiological studies our results suggest that the altered myogenic tone in BKβ1 -/- mice is not directly causing hypertension and that altered pressure-sensitive regulation of renal prorenin/renin secretion underlies the pathophysiology of hypertension in BKβ1 -/- mice.

5. Somato-dendritic voltage-gated A-type potassium channels and their modulatory subunits

Robert Bähring, Britta Callsen, Jan Ebbinghaus, Manuel Gebauer, Sven Hartmann, Dirk Isbrandt, Christian Legros*, Andreas Nolting*, Kathrin Sauter*

Kv4 voltage-gated potassium channels, associated with accessory subunits, mediate the transient outward current (I_{to}) in cardiac myocytes and the subthrethold A-type current (I_{SA}), which shows high densitiy in the dendrites of central neurons. In CA1 pyramidal neurons of the hippocampus Kv4.2 mainly represents the molecular substrate of I_{SA}, where it plays a significant role in dendritic integration and synaptic plasticity.

Using the whole-cell patch-clamp technique we found that the venom of the tarantula *Theraphosa leblondi* may selectively inhibit the A-type current component in cultured hippocampal neurons of postnatal mice. For a peptide toxin (TLTx1) isolated from this venom we showed that it acts as a gating-modifier of Kv4.2 channels. By binding to the voltage-sensor rather to the Kv4.2 pore domain, TLTx1 shifts the voltage for half-maximal activation to more positive potentials and influences Kv4.2 current kinetics. TLTx1 represents a novel member of a family of Kv4-specific gating-modifier toxins.

Coexpression of the accessory Ca^{2+}-binding Kv Channel Interacting Proteins (KChIPs) slows the onset of inactivation of Kv4.2-mediated currents and accelerates their recovery from inactivation. In addition, Kv4.2-mediated current densities are increased in the presence of KChIPs due to an increase in channel surface expression. We performed a scanning mutagenesis of regions within the Kv4.2 N-terminus known to be critically involved in Kv4.2/KChIP interaction, and created C-terminal deletion mutants of the Kv4.2 a-subunit. Combining the electrophysiological measurement of inactivation kinetics as well as current densities with coimmunoprecipitation experiments we identified both N- and C-terminal structural determinants for the functional Kv4.2/KChIP interaction and accessory subunit binding.

Kv4.2 channel inactivation is governed by a prominent cumulative closed-state inactivation, which is particularly evident in the presence of accessory KChIPs. Using different voltage-clamp techniques we showed that Kv4.2 channels do undergo open-state inactivation, mediated by an N-terminal inactivation domain. However, the interaction of KChIPs with the Kv4.2 N-terminus largely suppresses open-state inactivation. In addition, KChIPs enhance both Kv4.2 channel closure and transitions between closed and closed-inactivated states. Apparently, Kv4.2 channel properties are fine-tuned by accessory subunits in order to fulfill their dendritic function.

6. Frequenin

Jens Dannenberg, Birgit Grafelmann, Malte Stockebrand*

Like the KChIPs, Frequenin (Frq), also known as neuronal calcium sensor-1 (NCS-1), is a member of the family of neuronal calcium-sensor (NCS) proteins. KChIPs and Frq are close relatives. Frequenin has attracted much attention, because it may function as a calcium-sensor to modulate synaptic activity and secretion.

Frequenin, is an N-myristoylated Ca^{2+}-binding protein that has been conserved in both sequence and three-dimensional fold during evolution. We demonstrated using both genetic and biochemical approaches that the observed structural conservation between *Saccharomyces cerevisiae* frequenin (Frq1) and human NCS-1 is also reflected at the functional level. In yeast, the sole essential target of Frq1 is the phosphatidylinositol 4-kinase isoform, Pik1; both *FRQ1* and *PIK1* are indispensable for cell viability. Expression of human NCS-1 in yeast, but not a close relative (human KChIP2), rescues the inviability of *frq1* cells. Furthermore, *in vitro*, Frq1 and NCS-1 (either N-myristoylated or unmyristoylated) compete for binding to a small 28-residue motif near the N-terminus of Pik1. We propose, therefore, that the function of NCS-1 in mammals may closely resemble that of Frq1 in *S. cerevisiae* and, hence, that frequenins in general may serve as regulators of certain isoforms of phosphatidylinositol 4-kinase.

7. Pathophysiology underlying creatine deficiency disorders

D. Isbrandt, R. Peco, A. Neu, A. Schmidt**

Guanidinoacetate methyltransferase (GAMT) deficiency belongs to the family of “creatine deficiency disorders” and is an autosomal recessively inherited disease of creatine biosynthesis. It manifests during the first months of life with

severe neurological symptoms including developmental delay or arrest, ataxia and epilepsy. The neurological abnormalities observed in GAMT deficiency might be explained partially by the deficiency of high-energy phosphates in cells with high and fluctuating energy demands, while others appear to be related to the accumulation of the metabolite guanidinoacetate (GAA), which exhibits strong GABAergic properties when applied to neurons in acute murine brain slices.

In order to investigate the consequences of GAMT deficiency in mice, we generated a knockout mouse model by gene targeting in embryonic stem cells. Disruption of the open reading frame of the murine GAMT gene in the first exon resulted in the elimination of 210 of the 237 amino acids present in mGAMT. The creation of an mGAMT null allele was verified at the genetic, RNA, and protein levels. GAMT knockout mice have markedly increased guanidinoacetate (GAA) and reduced creatine and creatinine levels in brain, serum, and urine, which are key findings in human GAMT patients. *In vivo* ^{31}P magnetic resonance spectroscopy showed high levels of PGAA and reduced levels of creatine phosphate in heart, skeletal muscle, and brain. These biochemical alterations were comparable to those found in human GAMT patients and can be attributed to the very similar GAMT expression patterns found by us in human and mouse tissues. We provided evidence that GAMT deficiency in mice causes biochemical adaptations in brain and skeletal muscle. It is associated with increased neonatal mortality, muscular hypotonia, decreased male fertility, and a non-leptin-mediated life-long reduction in body weight due to reduced body fat mass. In conclusion, GAMT knockout mice are a valuable creatine deficiency model for studying the effects of high-energy phosphate depletion in brain, heart, skeletal muscle, and other organs.

8. Clinical research group

Patrick Friederich, Michael Chmielinski, Ulrike Eckhoff, Johnny Kim, Mark Andre Punke, Ralf Scholz, Cornelia Siebrands, Anna Solth

Hereditary and acquired alteration of potassium channel function is a well recognized cause of severe cardiac arrhythmia and neuronal disease such as epilepsy. Many clinically used therapeutic agents cause severe cardiac and neuronal side effects including cardiac arrhythmia and seizure. Our interdisciplinary research group consisting of basic scientist and clinicians focuses on identifying molecular mechanisms underlying hereditary as well as drug induced states of pathologically altered cardiac and neuronal excitability, in particular to study drug induced cardiac arrhythmia and drug induced seizure by investigating the interaction of local anaesthetics with HERG/MiRP1 and KCNQ2/Q3 channels.

Local anaesthetic induced cardiac arrhythmia

HERG channels underlie the repolarizing cardiac potassium current I_{Kr}. They are critical for the maintenance of normal rhythmicity in human heart. Mutations in the HERG gene may lead to dysfunctional HERG channels and a reduced I_{Kr}. This is correlated with the prolongation of ventricular action potentials as well as an increase in susceptibility to ventricular arrhythmia. Inhibition of HERG channels has also been associated with local anaesthetic induced long QT syndrome and ventricular fibrillation. HERG channels may associate in human myocardium with minK related peptide 1 (MiRP1) to form I_{Kr}. This auxillary subunit encoded in *KCNE2* has been reported to alter the pharmacological sensitivity of HERG

channels. Several mutations in *hKCNE2* as well as the common T8A polymorphisms of MiRP1 have been identified to predispose individuals to drug induced cardiac arrhythmia. Although our data supports the notion that local anaesthetics cause cardiac arrhythmia in part by interacting with HERG channels our results do not support the idea that MiRP1 or mutations in *hKCNE2* are factors involved in proarrhythmic drug action. Besides their cardiotoxic effects accidental intravenous injection, overdosage or rapid systemic uptake of local anesthetics may result in severe neurotoxic side effects such as seizures. By studying the interactions of bupivacaine with retigabine at human KCNQ2/Q3 channels we defined the possibility of a novel therapeutic approach in the treatment of local anesthetic induced seizures. This may be particularly advantageous as retigabine in contrast to commonly used agents for the treatment of local anesthetic induced seizure has not been reported to cause respiratory depression.

9. Research group synaptic plasticity

*Dietmar Kuhl**

The main goal of our research is to identify and study genes contributing to synaptic plasticity in the mammalian (mouse) brain. A range of genes has been found of which analysis of their expression and regulation indicates a relatively broad role in neuronal plasticity, including learning and memory, epilepsy, and mental diseases. The main forms of our research is the analysis of learning and memory. Much progress has been made, within discrete levels of analysis, characterizing biophysical, molecular and cellular adaptations associated with plasticity and cognitive functions. However, it has proven difficult to integrate these findings and translate the specific knowledge at each level into an understanding of information processing and storage. A long-term goal of our research is to elucidate how mental functions emerge from specific changes at molecular levels. We see the use of mouse genetics as an important means of building bridges between molecular biology and systems neurobiology and between systems neurobiology and behavior. This provides the rationale for an integrated approach to follow the flow of information from excitatory events in the dendrite through neuronal networks in behaving animals. We hope in this way to extract some of the fundamental rules that govern dendritic information processing in the activity-driven refinement of networks that underlies learning and memory.

In February 2002 the group moved to Berlin due to an appointment of D. Kuhl as full professor at the Free University of Berlin. His successor will be Ulrich Boehm, Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

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Publications

(1) R. Netzer, A. Ebneth, U. Bischoff and O. Pongs. (2001) Screening lead compounds for QT interval prolongation. *DDT*. 6, 78-84.

(2) E. Schulze-Bahr, M. Schwarz, S. Hoffmann, H. Wedekind, H. Funke, W. Haverkamp, G. Breithardt, O. Pongs and D. Isbrandt. (2001) A novel long-QT 5 gene mutation in the C-terminus (V109I) is associated with a mild phenotype. *J. Mol. Med.* 79, 504-509.

(3) H. Hu, L-R. Shao, S. Chavoshy, N. Gu, M. Trieb, R. Behrens, P. Laake, O. Pongs, H.G. Knaus, O. P. Ottersen and J. F. Storm. (2001) Presynaptic Ca^{2+}-activated K^{+} channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J. Neurosci.* 21, 9585-9597.

(4) D. Isbrandt, P. Friederich, A. Solth, W. Haverkamp, A. Ebneth, M. Borggrefe, H. Funke, K. Sauter, G. Breithardt, O. Pongs, E. Schulze-Bahr. (2002) Identification and functional characterization of a novel KCNE2 (MiRP1) mutation that alters HERG channel kinetics. *J. Mol. Med.* 80, 524-532.

(5) C. Legros, C. Schulze, M.L. Garcia, P.E. Bougis, M-F. Martin-Eauclaire, O. Pongs. (2002) Engineering specific pharmacological binding sites for peptidyl inhibitors of potassium channels into KcsA. *Biochemistry* 41, 15369-15375.

(6) P. Friederich, D. Benzenberg, B.W. Urban (2002) Bupivacaine inhibits human Kv3 channels. *Br. J. Anaesth.* 88: 864-866.

(7) P. Friederich, T. Schmitz (2002) Lidocaine induced cell death in a human model of neuronal apoptosis. *Eur. J. Anaesthesiol.* 19: 564-570.

(8) A. Neu, H. Neuhoff, G. Trube, S. Fehr, K. Ullrich, J. Roeper and D. Isbrandt. (2002) Activation of $GABA_A$ receptors by guanidinoacetate : a novel pathophysiological mechanism. *Neurobiology of Disease* 11, 298-307.

(9) P. Wulff, V. Vallon, D.Y. Huang, H. Volkl, F. Yu, K. Richter, M. Jansen, M. Schlunz, K. Klingel, J. Loffing, G. Kauselmann, M.R. Bosl, F. Lang, D. Kuhl. (2002) Impaired renal Na(+) retention in the sgk1-knockout mouse. *J Clin Invest.* 110; 1263-1268.

(10) S. Warntges, B. Friedrich, G. Henke, C. Duranton, P.A. Lang, S. Waldegger, R. Meyermann, D. Kuhl, E.J. Speckmann, N. Obermuller, R. Witzgall, A.F. Mack, H.J. Wagner, A. Wagner, S. Broer, F. Lang. (2002) Cerebral localization and regulation of the cell volume-sensitive serum- and glucocorticoid-dependent kinase SGK1. *Pflugers Arch.* 443, 617-624.

(11) E. Schulze-Bahr, A. Neu, P. Friederich, U. B. Kaupp, G. Breithardt, O. Pongs and D. Isbrandt. (2003) Pacemaker channel dysfunction in a patient with sinus node disease. *J. Clin. Invest.* 111, 1537-1545.

(12) M. Grunnet, T. Jespersen, N. Maculay, N. K. Jorgensen, N. Schmitt, O. Pongs, S.-P. Olesen and D. A. Klaerke (2003) KCNQ1 channels sense small changes in cell volume. *J. Physiol.* 549, 419-427.

(13)L. Conforti, K. Takimoto, M. Petrovic, O. Pongs and D. Millhorn. (2003) The pore region of the Kv1.2 α subunit is an important component of recombinant Kv1.2 channel oxygen sensitivity. *Biochem. Biophys. Res. Com.* 306, 450-456.

(14)S. Teng, L. Ma, Y. Zhen, C. Lin, R. Bähring, V. Vardanyan, O. Pongs, R. Hui. (2003) Novel gene hKCNE4 slows the activation of the KCNQ1 channel. *Biochem. Biophys. Res. Com.* 303, 808-813.

(15)M. A. Punke, T. Licher, O. Pongs and P. Friederich. (2003) Inhibition of human TREK-1 channels by bupivacaine. *Anest. Analg.* 96, 1665-1673.

(16)L. Ma, C. Lin, S. Teng, Y. Chai, R. Bähring, V. Vardanyan, L. Li, O. Pongs and R. Hui. (2003) Characterization of a novel Long QT syndrome mutation G52R-KCNE1 in a Chinese family. *Cardiovascular Research*. 59, 612-619.

(17)T. Strahl, B. Grafelmann, J. Dannenberg, J. Thorner and O. Pongs. (2003) Conservation of regulatory function in calcium-binding proteins: Human frequenin (neuronal calcium sensor-1) associates productively with yeast phosphatidylinositol 4-kinase isoform, Pik1. *J. Biol. Chem.* 278, 49589-49599.

(18)J. Ebbinghaus, C. Legros, A. Nolting, C. Guette, M.-L. Celerier, O. Pongs and R. Bähring. (2003) Modulation of Kv4.2 channels by a peptide isolated from the venom of the giant bird-eating tarantula *Theraphosa leblondi*. *Toxicon* (in press)

(19)P. Friederich, J.P. Dilger, D. Isbrandt, K. Sauter, O. Pongs, B.W. Urban. (2003) Biophysical properties of Kv3.1 channels in SH-SY5Y human neuroblastoma cells. *Receptors Channels*. 9, 387-396.

(20)P. Friederich (2003) Basic Concepts of Ion Channel Physiology and Anaesthetic Drug Effects. Eur. J. Anaesthesiol. 20: 343-353.

(21)W.K. Renema, A. Schmidt, J.J. Van Asten, F. Oerlemans, K. Ullrich, B. Wieringa, D. Isbrandt, A. Heerschap. (2003) MR spectroscopy of muscle and brain in guanidinoacetate methyltransferase (GAMT)-deficient mice: Validation of an animal model to study creatine deficiency. *Magn Reson Med*, 50, 936-943.

(22)M. Madeja, T. Leicher, P. Friederich, M.A. Punke, W. Haverkamp, U. Mußhoff, G. Breithardt, E.J. Speckmann (2003) Molecular site of action of the antiarrhythmic drug propafenone at the voltage-operated potassium channel Kv2.1. *Mol. Pharmacol.*, 63: 547-556

(23)R. Bähring, V. Vardanyan and O. Pongs. (2004) Differential modulation of Kv1 channel-mediated currents by coexpression of Kvβ3 subunit in a mammalian cell-line. *Mol. Memb. Biol.* 21: 19-25.

(24)S. Teng, L. Ma, Y. Dong, C. Lin, J. Ye, R. Bahring, V. Vardanyan, Y. Yang, Z. Lin, O. Pongs, R. Hui. (2004) Clinical and electrophysiological characterization of a novel mutation R863X in HERG C-terminus associated with long QT syndrome. *J. Mol. Med.* (published on line)

(25)H. Wedekind, S. Hauenschild, M. Schwarz, D. Isbrandt, H. Djonlagic, T. Wülfing, O. Pongs, G. Breithardt, W. Haverkamp, E. Schulze-Bahr. (2004) Long-term follow-

up of a family with Romano-Ward syndrome and two KCNQ1 mutations located in cis. *Clin. Genet.* 65: 233-241.

(26)M. Gebauer, D. Isbrandt, K. Sauter, B. Callsen, A. Nolting, O. Pongs and R. Bähring. (2004) N-type inactivation features of Kv4.2 channel gating. *Biophysic. J.* 86: 210-223

(27)M. J. Sinneger-Brauns, A. Hetzenauer, I. G. Huber, E. Renström, G. Wietzorrek, S. Berjukov, M. Cavalli, D. Walter, R. Waldschütz, S. Hering, S. Bova, P. Rorsman, O. Pongs, N. Singewald and J. Striessnig. (2004) Cav1.3 L-type calcium-channels: role for isoform-specific regulation of mood behavior, pancreatic beta-cell and cardiovascular function. *J. Clin. Invest.* (in print)

(28)P. Friederich, A. Solth, S. Schillemeit, D. Isbrandt. (2004) Local anaesthetic sensitivities of cloned HERG channels from human heart: comparison with HERG/MiRP1 and HERG/MiRP1 T8A. Br. J. Anaesth. 92: 93-101.

(29)M.A. Punke, P. Friederich (2004) Retigabine stimulates KCNQ2/Q3 channels in the presence of bupivacaine. *Anesthesiology* (Accepted for publication)

(30)A. Schmidt, B. Marescau, E.A. Boehm, W.K. Renema, R. Peco, A.M. Das, R. Steinfeld, S. Chan, J. Wallis, M. Davidoff, K. Ullrich, R. Waldschutz, A. Heerschap, P.P. DeDeyn, S. Neubauer, D. Isbrandt (2004) Severely altered guanidino compound levels, disturbed body weight homeostasis, and impaired fertility in a mouse model of guanidinoacetate N-methyltransferase (GAMT) deficiency. *Hum Mol Genet*, 13, 1-17.

Reviews

O. Pongs (2001) Molecular Biology of Voltage-gated K^+ Channels. Potassium *Channels in Cardiovascular Biology* (Eds. S. Archer and N. Rusch, Kluwer Acad./ Plenum Publ., New York) 35-48.

O. Pongs (2001) Classification and Structure of Ion Channels. *The Headaches* (Eds. J. Olesen, P. Teelt-Hansen, K.M. Welch; Lippincott Williams and Wilkins Publ., New York) 151-162.

Dissertations

Susan Hauenschild: Untersuchungen von Mutationen in Kaliumkanalgenen von Patienten mit LQT-Syndrom (2002)

Thomas Licher: HTS-Testverfahren für Ionenkanal-modulierende Substanzen (2002)

Axel Gustav Neu: Elektrophysiologische Untersuchungen an r-elk- und HCNA-Kanälen (2002)

Howard Christian Peters: Transgene induzierbare Expression von dominant-negativen KCNQ2 Kaliumkanal-Untereinheiten im Gehirn der Maus (2002)

Manuel Gebauer: Untersuchung der Inaktivierung des humanen Kv4.2-Kanals und der Effekte von Kv-Kanal-interagierenden Proteinen (KChIPs) (2003)

Andreas Nolting: Untersuchungen zur Toxizität von Blei am Beispiel der Effekte von Pb^{2+} auf den neuronalen spannungsabhängigen Kaliumkanal Kv1.1 (2003)

Oliver Michael Steinmetz: Differentielle Expression von Spleißvarianten und potentieller neuer Interaktionspartner zur Heteromultimerbildung von BK-α im ZNS der Maus (2003)

Vitya Vardanyan: Structural requirements and role of oxidoreductase features for Kvβ-mediated potassium channel inactivation (2003)

Diplomas

John Kim: Elektrophysiologische und pharmakologische Untersuchungen des humanen Ionenkanalkomplexes HERG/14-3-3e (2003)

Ralf Scholz: Elektrophysiologische und pharmakologische Charakterisierung neonataler hippokampaler Neurone der Maus und Etablierung eines akuten Epilepsie Modells (2004)

Habilitations

Robert Bähring: Spannungsgesteuerte A-Typ K^+-Kanäle und ihre akzessorischen Unereinheiten (2003)

Patrick Friederich: Humane Kv Kanäle als *in-vitro* Modell zur Bewertung konvulsiver Eigenschaften von Anästhetika (2002)

Prices

Dirk Isbrandt: Bickel award of the German Cardiological Society (2002)

Axel Neu: Finkelstein-Award of the North German Pediatric Society (2002)

Patrick Friederich: Research Award of the European Society of Anesthesiologists (2003)

Appointments

Prof. Dr. Dietmar Kuhl, Molecular Neurobiology, Institute of Biology, Department of Chemistry, Pharmacology, and Biology, Free University of Berlin.

Collaborations

Prof. Bernard Attali, Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel.

Prof. Dr. K. Benndorf, Institute of Physiologiy, University of Jena, Germany

Dr. P. E. Bougis, CNRS, Institute Jean Roche – University of the Mediterranean, Marseille, France.

Dr. Y. Bourne, AFMB CNRS, Marseille, France.

Prof. Dr. A. Breithardt, University of Münster, Germany.

Dr. H-G. Knaus, Institute of Biochemical Pharmacology, Innsbruck, Austria.

Dr. P. Marchot, CR1-CNRS, Jean Roche Research Institute, University of the Mediterranean, Marseille, France.

Dr. M-F. Martin-Eauclaire, Jean Roche, University of the Mediterranean, Marseille, France.

Prof. Dr. J. S. Storm, Institute of Physiology, Oslo, Norway.

Collaborations with colleagues at the University Clinics Hamburg

Prof. Dr. A. Engel, Neurophysiology

Prof. Dr. H. Ehmke, Physiology

Prof. Dr. T. Münzel, Cardiology

Prof. Dr. Jürgen R. Schwarz, Physiology

Prof. Dr. J. Schulte am Esch, Anaesthesiology

Prof. Dr. K. Ullrich, Pediatry

Prof. Dr. C. Wagener, Clinical Chemistry

Structure of the Institute

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	Dr. Birgit Engeland*
	Dr. C. Legros*
	Dr. Dirk Isbrandt
	Dr. Axel Neu*
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	Birgit Grafelmann
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	Quyen Le
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	Lijuan Ma
	Andreas Nolting *
	Christoph Ogrodowczyk
	Ruben Peco

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	Lars Schlichting*
	Malte Stockebrand
	Vitya Vardanyan*
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	Birgit Hantzsch
	Oliver Steinmetz
Clinical research group	
Group leader:	Dr. Patrick Friederich
Postdoctoral fellow:	Dr. Mark A. Punke
Graduate students:	Michael Chmielinski
	Ulrike Eckhoff
	Johnny Kim*
	Ralf Scholz
	Cornelia Siebrands
	Anna Solth

Junior research group

Group leader:	Dr. Dietmar Kuhl*

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Institut für Zellbiochemie und Klinische Neurobiologie

Dietmar Richter

The main aim of research in this institute is to understand how nerve cells manage to respond to external and internal signals in order to maintain and regulate their cellular architecture. (i) External signalling is studied, using as tools, neuropeptide hormone receptors as well as proteins of the taste receptor family. The expression patterns of these proteins, the delineation of their ligand binding sites as well as their structural requirements for intracellular signalling are examined in mammals. (ii) As an example for internal signalling, the molecular mechanisms underlying selective cytoplasmic mRNA trafficking into dendrites and axons are investigated. Decentralized local protein synthesis may govern the spatial organization of complex protein repertoires and, thus, may be critical for the generation and maintenance of pattern, polarity and plasticity in nerve cells. Cis-elements and trans-acting factors that are involved in the subcellular targeting of mRNAs within neurons are presently being investigated.

1. Neurotransmitter receptors and their associated signalling proteins

Hans-Jürgen Kreienkamp, Kerstin Berhörster, Agata Blasczcyk-Wewer, Marcus Christenn*, Günter Ellinghausen*, Hans-Hinrich Hönck, Peter Iglauer*, Marie G. Mameza*, Chong Wee Liew, Arne Quitsch*, Mailin Segger-Junius*, Michaela Soltau*, Wolf Wente*

Signal transduction processes by neurotransmitter receptors are mediated by a series of defined protein-protein interactions. Many proteins that physically interact with transmitter receptors have been, and continue to be identified. The functions of these protein interactions appear to be (i) the targeting of receptors to cellular specializations (e.g. the postsynaptic compartment in a neuron) (ii) anchoring of receptors to the cytoskeleton and (iii) enabling a tight association with elements of a receptor-specific signal transduction machinery, thus providing specificity as well as increased speed and accuracy in signal.

Starting from the C-terminal, intracellular regions of G-protein coupled somatostatin receptors (SSTR1-5) we have now identified specific interacting proteins for each receptor subtype. Some interacting partners, such as the PDZ domain protein PIST, presumably have a function in the membrane targeting of SSTR3 and SSTR5. Others such as the tight junction protein MUPP1 or the postsynaptic scaffolds PSD-95 (interacting with SSTR4) or SSTRIP/shank (SSTR2) link receptors into very large signalling complexes, such as the postsynaptic density in excitatory synapses of the central nervous system.

We have further focused on shank proteins and their role in the assembly of the postsynaptic receptor complex in neruons. Besides direct and indirect interactions with transmitter receptors and actin binding proteins, we have identified an interaction with the insulin receptor substrate of 53 kDa (IRSp53) which is regulated by the small G-protein cdc42. IRSp53 links shank to another prominant postsynaptic scaffold, PSD-95, via a PDZ-type interaction. In neurons, overexpressed, IRSp53 induces the formation of dendritic filopodia and the branching of dendrites in the absence of shank. In the presence of shank, IRSp53 instead contributes to assembly of postsynaptic shank complexes. Our data suggest that IRSp53 is a platform for cdc42-

mediated signalling during neuronal morphogenesis and synaptogenesis.

A further interaction of the SH3 domain of shank was identifed with the leucine-rich-repeat and PDZ domain containing protein densin. In neurons, densin induces increased denritic branching. As seen before with IRSp53, coexpressed shank blocks branching and recruits densin to the postsynaptic complex where bot proteins colocalize.

In collaboration with the group of Tobias Böckers (now University of Ulm, Germany) we have shown that all three shank mRNAs are present in neuronal dendrites. Dendritic targeting of the shank1 mRNA is mediated by a small 200 base targeting element in the 3' untranslated region of shank1. We currently assume that shank mRNAs are translocated to dendrites in a translationally silent state; upon an unidentified signal, shank mRNAs will be translated locally and will contribute to the functional matu-ration of the postsynaptic protein complex.

2. Sweet taste receptors and their intracellular interacting proteins

Hartwig Schmale, Nicole Burhenne, Heidje Christiansen, Anette Henkel*, Jan K. Hennigs*, Irina Kempel* , Bettina Walter**

The sense of taste contributes to food palatability, which promotes food intake and to sensory-specific satiety, which promotes termination of intake. Reduced sensitivity to these sensory-based signals may contribute to the over-consumption of energy that leads to obesity. In order to prove the hypothesis that taste has an impact on food preference and eating behaviour and therefore may be one factor associated with the development and progression of human eating disorders it is essential to understand basic mechanisms of sweet taste transduction.

Taste perception is mediated by defined transduction pathways involving ion channels and membrane receptors of taste cells present in taste buds on the tongue and palate. Taste stimuli such as sugars, artificial sweeteners, amino acids including glutamate and several bitter compounds bind to G-protein-coupled taste receptors thereby activating different intracellular second messenger systems. Two families of receptors, T1R -sweet and -umami receptors and T2R -bitter receptors, have been identified. Sweet receptor subunits form heterodimers in the combinations T1R1+T1R3 and T1R2+T1R3, which are activated by L-amino acids and various sweet compounds, respectively. In related systems, the importance of protein-protein interactions of intracellular receptor domains with various adapter molecules mediating receptor localization, cytoskeletal anchoring and coupling to signal transduction components was demonstrated. Proteins participating in a "signalosom" of sweet receptors have not been reported up to now

To identify proteins involved in the signalling pathways of T1Rs we have used the yeast two-hybrid system to screen a human keratinocyte cDNA library with the C-terminal domains of rat T1R1 and T1R2 as "baits". The Calcium Integrin-binding protein CIB/calmyrin showed strong and specific binding activity towards the C-terminal 29 amino acids of rT1R2. Calmyrin is expressed in rat taste tissue as determined by RT-PCR and Northern Blot analyses. GST-pulldown assays were used to verify the direct binding of rT1R2ct to calmyrin. Further evidence for the interaction was provided by co-immunoprecipitation and co-localization

studies after expression of rT1R2ct and calmyrin in HEK293 cells. Binding activity of calmyrin to rT1R2ct is greatly diminished by substituting valine for methionine$_{818}$ in the membrane-proximal part of the receptor C-terminal domain.

Like other members of the EF-hand superfamily calmyrin belongs to the family of Ca^{2+} -myristoyl switches that probably regulate effector molecules at the membrane in a calcium-dependent manner. We could show the redistribution of myristoylated calmyrin from cytoplasmic to membrane compartments upon Ca^{2+} increase in HEK293 cells. Calmyrin has similarity to the regulatory subunit calcineurin B of the phosphatase calcineurin and was found to interact with various proteins including kinases and proteinases. In sweet taste transduction, calmyrin may be involved in desensitisation events mediated by kinases and phosphatases at the receptor C-terminal domain.

3. Dendritic mRNA targeting and the genesis and plasticity of synaptic signalling complexes

Stefan Kindler, Monika Rehbein, Cornelia Brendel, John Jia En Chua, Marina Walden*, Konstanze Wege*, Krishna H. Zivraj*, Christiane Schröder-Birkner, Birgit Schwanke*

Neurons possess distinct cellular compartments that are highly diverse with respect to their protein repertoires. In particular, synapses serving as communication sites between neurons are equipped with a highly specialized set of molecules. Synaptic plasticity underlying learning and memory involves a synapse-specific modification of the protein composition. This adaptation is established by two cellular mechanisms, namely, synaptic targeting of somatically-synthesized proteins and extrasomatic protein synthesis near synapses. The small group of dendritic mRNAs includes transcripts encoding the microtubule-associated protein 2 (MAP2) and the α subunit of the Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII). To functionally characterize *cis*-acting dendritic targeting elements (DTEs) in both messages we have expressed tagged MAP2 and CaMKII mRNA fragments in cultured primary neurons. DTEs are situated in the 3'-untranslated regions of both transcripts. Two 90- and 65-kDa MAP2-RNA *trans*-acting proteins, MARTA1 and MARTA2, specifically interact with the MAP2-DTE. Both proteins belong to the family of FUSE-binding proteins and possess four central KH domains, which mediate RNA-binding. In neurons, MARTA1 and MARTA2 are found in nuclei, somata and along dendrites. Furthermore, two rat homologues of the *Drosophila* RNA-binding protein Staufen, named Staufen 1 and Staufen 2, were identified. In neurons, both proteins, which contain several double-stranded RNA-binding domains, are present in somata and dendrites. Distinct Staufen 1 isoforms exhibit different RNA-binding capacities and interact with protein phosphatase 1 and ribosomes.

The four members of the family of synapse-associated protein 90/postsynaptic density-95-associated proteins, SAPAP1-4, are adapter proteins of the postsynaptic density (PSD). They interact with different synaptic scaffolding proteins, cytoskeletal and signalling components, and are therefore considered to assemble functional multi-protein units at synapses. We have shown that all four SAPAP genes are expressed in many regions of the postnatal rat brain leading to overlapping yet distinct mRNA distribution patterns. In the hippocampus, SAPAP 1, 2 and 4 transcripts

are restricted to cell body zones, whereas SAPAP3 mRNAs are also detected in molecular layers. Thus, SAPAP3 is one of the few PSD components whose activity-dependent local synthesis in dendrites may directly contribute to an input-specific adaptation of dendritic spine function.

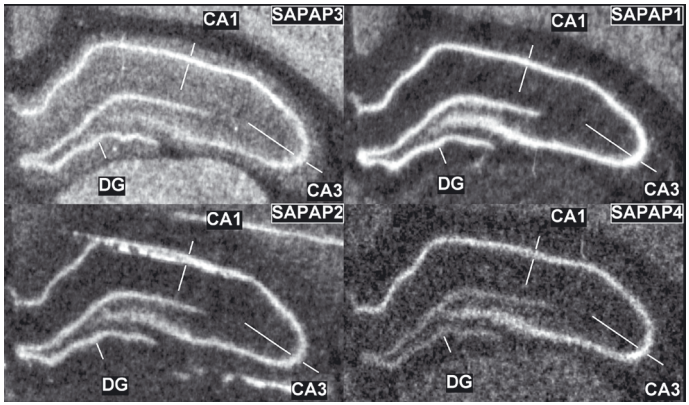

Figure 1. Distinct distribution of SAPAP1-4 mRNAs in the hippocampal area of the adult rat brain as determined by *in situ* hybridization of frontal sections. SAPAP3 transcripts are detected in the molecular layers (marked by white lines) of the dentate gyrus (DG) and hippocampal CA1 and CA3 regions.

4. Intracellular vasopressin mRNA transport

Evita Mohr, Carola Mullin, Hatmone Miroci*, Stefanie Reinhardt**

Specific sorting of a subset of cellular mRNAs to defined cytoplasmic regions takes place in different organisms and cell types including neurons. Nerve cells harbor a still growing number of different mRNA species in dendrites and, infrequently, in the axon. mRNA transport is specified by molecular entities encompassing distinct sequence elements within the RNA molecule (*cis*-acting elements) and proteins (*trans*-acting factors) which associate with those elements to build up a complex transport machinery. The current line of thinking is that proteins with key functions in synaptic plasticity are, at least in part, produced by local dendritic translation of their mRNAs.

We have shown earlier that the mRNA encoding the rat vasopressin (VP) precursor is sorted to axons and dendrites of hypothalamic magnocellular neurons. Magnocellular neurons, like many other nerve cell types, are capable of synthesizing cytosolic as well as membrane-bound and secretory proteins within their dendrites while the axonal compartment appears to lack protein synthesizing capacity. VP is secreted from the nerve terminals into the systemic circulation. However, substantial amounts are also released from the dendrites of magnocellular neurons into the brain. This raises the question as to whether the dendritic portion of the precursor originates, at least in part, from local translation. When expressed in cultured nerve cells from superior cervical ganglia (SCG) VP transcripts are delivered to dendrites while axonal targeting of the mRNA does not appear to take place (or is beyond the level of detection). In order to determine whether the VP precursor is produced in the dendrites of SCG neurons we have made use of a naturally occurring mutant version of this secretory protein. In the Brattleboro (BB) rat a frameshift mutation in the VP precursor-encoding gene leads to synthesis of a protein with an altered amino acid sequence of its C-terminal part which renders it incapable of leaving the site of its synthesis, the rough endoplasmic reticulum (RER). In SCG neurons microinjected with an appropriate expression vector the RER-bound BB rat VP precursor was restricted to those parts of the dendrites that also contained the corresponding mRNA indicating on-site synthesis of the mutant protein.

In vitro protein/RNA-interaction studies with rat brain cytosolic extracts demonstrated specific binding of a protein to a segment within VP mRNA that is required for dendritic sorting. Biochemical purification revealed that this protein is the multifunctional poly(A)-binding protein (PABP). It is well-known for its ability to bind with high affinity to poly(A) tails of mRNAs, prerequsite for stimulation of translational initiation. With lower affinities, PABP also associates with non-poly(A) sequences. The physiological consequences of these PABP/RNA interactions include functions such as translational control, mRNA stabilization/destabilization and presumably mRNA localization. The translational state of mRNAs subject to dendritic sorting appears to be influenced by external stimuli. PABP could, thus, be a component required to regulate on-site synthesis within dendrites of the VP precursor and possibly of other proteins.

5. Neuropeptide receptors and regulation of mammalian food intake behavior

Dietmar Bächner, Felix Francke, Vanessa Schröder**

Food intake is a complex behavior regulated by a large number of peripheral and central signals. Orexigenic hormons induce food intake behavior in response to energy deficiency and anorexigenic hormones, produced in response to food intake, act as satiety signals. Understanding the regulation of appetite is becoming increasingly important as overweight and its morbid form obesity is increasing worldwide, with adverse consequences for human health.

Our work focuses on the signal transduction of the Melanin-concentrating hormone, MCH, a key neuropeptide hormone controlling food intake and energy homeostasis. Using a reverse-pharmacological approach, we identified the endogenous MCH-receptor, MCH-R1, and showed coupling to Gi/o and Gq-type G-proteins. The MCH-R1 interacting zinc-finger protein (MIZIP), and Neurochondrin (NCDN), a neurite outgrowth promoting factor, were identified as C-terminal interacting proteins. The cellular functions of NCDN and MIZIP are unknown. Both induce an increase in 125J-MCH binding sites at the plasma membrane of transfected cells and are colocalized with MCH-R1 in vivo (Fig. 1). In addition, we detected a specific interaction of MIZIP with tubulin. Our present results suggest that MIZIP and NCDN may be important for the specific transport of MCH-R1 to the plasma membrane and that MIZIP may link the receptor to the microtubular cytoskeleton. To verify this hypothesis in vivo, we currently produce mouse strains deficient in MIZIP and NCDN.

6. Biochemical Analytics Group

Friedrich Buck, Sönke Harder, Jessica Heinze

The core competence of the lab is the analysis of DNA and proteins, using state-of-the-art technologies, in particular mass spectrometry techniques.

DNA Analysis: Since 2003 the lab is equipped with a modern capillary electrophoresis based DNA sequenator (ABI 3100) in addition to a sequenator using the traditional gel electrophoresis based technique (ABI 377). While the capillary electrophoresis technique provides for faster and more sensitive DNA sequencing, the gel electrophoresis is still used for specialized applications, e.g. microsatellite

analysis. For the analysis of short oligonucleotides, laser desorption (MALDI) mass spectrometry has been successfully applied.

Protein/Proteom Analysis: Modern protein analytics relies almost exclusively on mass spectrometric techniques. The lab is equipped with an electrospray tandem mass spectrometer (QTOF II, Micromass) and, since 2003, with a laser desorption (MALDI) mass spectrometer (Reflex IV, Bruker). Both techniques are complementary to each other: MALDI measurements allow the rapid screening of large numbers of samples, while the more elaborate and time consuming ESI tandem mass spectrometry allows the thorough characterization of complex samples. Even more detailed information on complex samples can be obtained by coupling capillary LC on line with the ESI tandem mass spectrometer. For special applications (e.g. de novo sequencing or exact determination of N-terminal sequences) the traditional Edman degradation technique is still available.

7. Analysis of differentiation factors in the nervous system

Stefan Schumacher, Veronika Schoop, Burkhard Hassel, Matthias Schreff*, Bettina Kirchner*, Eva-Maria Stübe*

Our work focuses on the functional analysis of two transmembrane proteins, which are prominently expressed in the nervous system and the expression of which is regulated during brain development. One of them, CALEB, is a member of the EGF family. The other protein, Mas, is a putative G protein-coupled receptor.

CALEB

CALEB was formerly shown to be expressed in synapse- and axon-rich areas in the nervous system, and experimental data indicated that CALEB may be important for neurite formation. To get insight into the molecular network CALEB is involved in, we searched for extracellular and cytoplasmic interaction partners of CALEB. Tenascin-C and tenascin-R were described as extracellular matrix proteins, which are able to bind to CALEB. We analysed these interactions in detail and found them to be regulated during development. Only CALEB-80, the expression of which is upregulated during developmental stages in the retina, when the greater part of synaptogenesis occurs, is able to bind the tenascins. In contrast, CALEB-140, the expression of which is high in the adult, does not bind to tenascin-C or –R.

Currently we try to elucidate the functional consequences of these regulated interactions for synaptogenesis.

One intracellular interaction partner of CALEB is PIST, a Golgi-associated protein, which had been shown to regulate the transport of several proteins from the Golgi apparatus to the plasma membrane. So far, we could not show that PIST is important for the general transport of CALEB to the plasma membrane. However, we present evidence that PIST may regulate the transport of CALEB from the cell body to the neuronal processes during nerve cell differentiation.

A subunit of the protein phosphatase 2A (PP2A) was found as a second cytoplasmic interaction partner of CALEB. With affinity chromatography and mass spectrometry we could demonstrate that CALEB is able to recruit the whole PP2A trimer via this subunit. Because the PP2A is involved in different aspects of neuronal cell differentiation, we are

currently underway to elucidate the function of this interaction with respect to the regulation of the neuronal cytoskeleton.

For the functional analysis of CALEB on the cellular level, we use different model systems. We examine the potential of CALEB to regulate the differentiation state of heterologous cells in culture and of primary neural cells derived from different parts of the brain. Our preliminary results indicate that CALEB influences neuronal differentiation via regulation of distinct cytoskeletal rearrangements at specific time points of development.

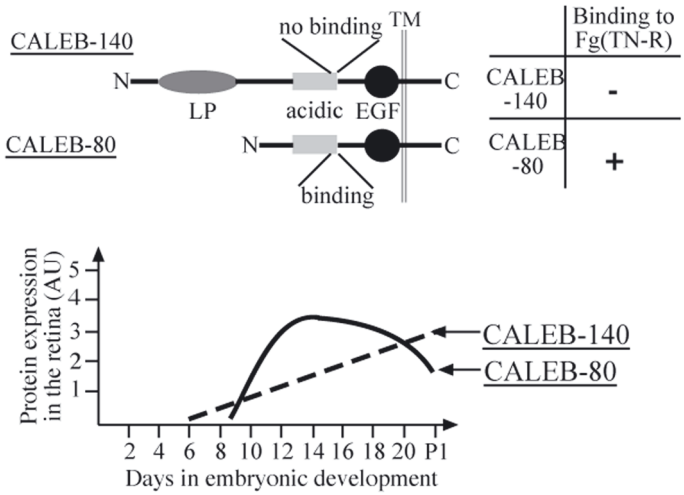

Figure 2. Comparison of CALEB-80 and CALEB-140 with respect to EFn-(TN-R) binding. A short summary of the binding results of the microsphere assay concerning CALEB-140 and CALEB-80 is shown in addition to schematic drawings of CALEB-140 and CALEB-80. The bottom scheme indicates the different regulations of expression of CALEB-140 and CALEB-80 in the embryonic chicken retina.

Mas

Mas is strongly expressed in the brain, in particular in the hippocampus. Despite extensive genetic research, the function of Mas in the nervous system remains unclear. We started to analyse Mas at the molecular and cellular level. Preliminary data point to the view that Mas is linked to several proteins of the postsynaptic density (PSD) and may indirectly interact with a novel class of proteins clinically relevant because of their involvement in epilepsy.

Support

The work in our institute is supported by the Deutsche Forschungsgemeinschaft, the Human Frontier Science Program Organization, the VW Stiftung, the EU.

Publications

(1) Bächner, D., Kreienkamp, H.-J. and Richter, D. (2002) MIZIP, a highly conserved, vertebrate specific Melanin-concentrating hormone receptor 1 interacting zinc-finger protein. FEBS Lett. 526, 124-128.

(2) Bächner, D., Schröder, D. and Gross, G.(2002) mRNA expression of the murine glycoprotein Nmb (Gpnmb) gene is linked to the developing retinal pigment epithelium and iris. Brain Res. Gene Expr. Patt. 1, 159-165.

(3) Bamberger, C., Pollet, D. and Schmale, H. (2002) Retinoic acid inhibits downregulation of ΔNp63α

expression during terminal differentiation of human primary keratinocytes. J. Invest. Dermatol. 118, 133-138.

(4) Böckers, T.M., Segger-Junius, M., Iglauer, P., Bockmann, J., Gundelfinger, E.D., Kreutz, M.R., Richter, D., Kindler, S. and Kreienkamp, H.-J. (2004) Differential expression and dendritic transcript localization of Shank family members: Identification of a dendritic targeting element in the 3' untranslated region of Shank1 mRNA. Mol. Cell. Neurosci., (in press).

(5) Casini, C., Dal Monte, M., Petrucci, C., Gambellini, G., Grouselle, D., Allen, J.P., Kreienkamp, H.-J., Richter, D., Epelbaum, J. and Bagnoli, P. (2004) Altered morphology of rod bipolar cell axonal terminals in the retinas of mice carrying genetic deletion of somatostatin subtype receptor 1 or 2. Eur. J. Neurosci., 19, 43-54.

(6) Coy, J. F., Wiemann, S., Bechmann, I., Bächner D., Nitsch, R., Kretz, O., Christiansen, H. and Poustka, A. (2002) Pore membrane and/or filament interacting like protein 1 (POMFIL1), is predominantly expressed in the nervous system and encodes different protein isoforms. Gene, 290, 73-94.

(7) Dietz, S., Rother, K., Bamberger, C., Schmale, H., Mössner, J. and Engeland, K. (2002) Differential regulation of transcription and induction of programmed cell death by human p53 family members p63 and p73. FEBS Lett. 525, 93-99.

(8) Hassel, B., Schreff, M., Stübe, E.-M., Blaich, U. and Schumacher, S. (2003) CALEB interacts with the Golgi-associated protein PIST (2003). J. Biol. Chem. 278, 40136-40143.

(9) Heidebrecht, H.-J., Adam-Klages, S., Szczepanowski, M., Pollmann, M., Buck, F., Endl, E., Kruse, M.-L., Rudolph, P. and Parwaresch, R. (2003) repp86: A human protein associated in the progression of mitosis. Mol. Cancer Res. 1, 271-279.

(10) Hoffmann, A., Czichos, S., Kaps, C., Bächner, D., Mayer, H., Zilberman, Y., Turgeman, G., Pelled, G., Gross, G. and Gazit, D. (2002) The T-box transcription factor Brachyury mediates cartilage development in mesenchymal stem cell line C3H10T1/2. J. Cell. Sci. 115, 769-781.

(11) Ignatov, A., Lintzel, J., Hermans-Borgmeyer, I., Kreienkamp, H.-J., Joost, P., Thomsen, S., Methner, A. and Schaller, C. (2003) Role of GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J. Neurosci. 23, 907-14.

(12) Ignatov, A., Lintzel, J., Kreienkamp, H.-J. and Schaller, C. (2003) Sphingosine-1-phosphate is a high-affinity ligand for the G protein-coupled receptor GPR6 from mouse and induces intracellular Ca2+ release by inducing the sphingosine-kinase pathway. Biochem. Biophys. Res. Commun. 311, 329-336.

(13) Kindler, S. and Monshausen, M. (2002) Candidate RNA-binding proteins regulating extrasomatic mRNA targeting and translation in mammalian neurons. Mol. Neurobiol. 25, 149-165.

(14)Kindler, S., Rehbein, M., Classen, B., Richter, D. and Böckers, T.M. (2004) Distinct spatiotemporal expression of SAPAP transcripts in the developing rat brain: a novel dendritically localized mRNA. Mol. Brain Res. (in press).

(15)Kreienkamp, H.J. (2002) Organization of G-protein mediated signalling complexes by scaffolding proteins. Curr. Opin. Pharmacology 2, 581-586.

(16)Kreienkamp, H.-J., Soltau, M., Richter, D. and Böckers, T. (2002) Interaction of G-protein coupled receptors with synaptic scaffolding proteins. Biochem. Soc. Transactions, 30, 464-468.

(17)Kreienkamp, H.-J., Larusson, H.J., Witte, I., Roeder, T., Birgül, N., Hönck, H.-H, Harder, S., Ellinghausen, G., Buck, F. and Richter, D. (2002) Functional annotation of two orphan G-coupled receptors, Drostar1 and-2, from Drosophila melanogaster and their ligands by reverse pharmacology. J.Biol.Chem. 277, 39937-39943.

(18)Mohr, E., Kächele, I., Mullin, C. and Richter, D. (2002) Rat vasopressin mRNA: a model system to characterize *cis*-acting elements and *trans*-acting factors involved in dendritic mRNA sorting. Prog. Brain Res. 139, 211-224.

(19)Mohr E. and Richter, D. (2002) In Encyclopedia of Molecular Medicine, ed. Creighton, T.E. (John Wiley & Sons, Inc., New York) Vol 5, pp 2250-2253 (2002). Neuropeptides and receptors.

(20)Mohr E. and Richter, D. (2003) RNA localization, subcellular: implications for cell polarity. Encyclopedia of the Human Genome.

(21)Mohr, E. and Richter, D. (2003) Local synthesis of the rat vasopressin precursor in dendrites of in vitro cultured nerve cells. Mol. Brain Res. 114, 115-122.

(22)Mohr E. and Richter, D. (2003) Molecular determinants and physiological relevance of extrasomatic RNA localization in neurons. Front. Neuroendocrinol. 24, 128-139.

(23)Mohr, E. and Richter, D. (2004) Subcellular VP mRNA trafficking and local translation in dendrites. J. Neuroendocrinol., (in press).

(24)Monshausen, M., Rehbein, M., Richter, D. and Kindler, S. (2002) The RNA-binding protein Staufen from rat brain interacts with protein phosphatase-1. J. Neurochem. 81, 557-564.

(25)Monte, M.D., Petrucci, C., Vasilaki, A., Cervia , D., Grouselle, D., Epelbaum , J., Kreienkamp H.-J., Richter, D., Hoyer, D. and Bagnoli, P. (2003) Genetic deletion of somatostatin receptor 1 alters somatostatinergic transmission in the mouse retina. Neuropharmacology 45, 1080-1092.

(26)Nalaskowski, M.M., Bertsch, U., Fanick, W., Stockebrand, M.C., Schmale H. and Mayr, GW. (2003) Rat inositol 1,4,5-trisphosphate 3-kinase C is enzymatically specialized for basal cellular inositol trisphosphate phosphorylation and shuttles actively between nucleus and cytoplasm. J. Biol. Chem. 278, 19765-76.

(27) Obermeyer, N., Janson, N., Bergmann, J., Buck, F. and Ito, W. (2003) Proteom analysis of migrating versus nonmigrating rat heart endothelial cells reveals distinct expression patters. Endothelium 10, 167-178.

(28) Pohl, S., Darlison, M.G., Clarke, W.C., Lederis, K. and Richter D. (2001) Cloning and functional pharmacology of two corticotropin-releasing factor receptors from a teleost fish. Eur. J. Pharmacol. 430, 193-202.

(29) Rehbein, M., Wege, K., Buck, F., Schweizer, M., Richter, D. and Kindler, S. (2002) Molecular characterization of MARTA1, a protein interacting with the dendritic targeting element of MAP2 mRNAs. J. Neurochem. 82, 1039-1046.

(30) Sarret, P., Esdaile, M.J., McPherson P.S., Schonbrunn, A., Kreienkamp, H.-J. and Beaudet, A. (2004) Role of amphiphysin II in somatostatin receptor trafficking in neuroendocrine cells. J. Biol. Chem., (in press).

(31) Schumacher, S. and Stübe E.-M. (2003) Regulated binding of the fibrinogen-like domains of Tenascin-R and Tenascin-C to the neural EGF family member CALEB. J. Neurochem. 87, 1213-1223.

(32) Soltau, M., Richter, D. and Kreienkamp, H.-J. (2002) The insulin receptor substrate IRSp53 links postsynaptic shank1 to the small G-protein cdc42. Mol. Cell. Neurosci. 21, 575-583.

(33) Videau, C., Hochgeschwender, U., Kreienkamp, H.-J., Brennan, M.B., Viollet, C., Richter, D. and Epelbaum, J. (2003) Characterisation of [125I]-Tyr0DTrp8-somatostatin binding in sst1- to sst4- and SRIF-gene-invalidated mouse brain. Naunyn Schmiedebergs Arch. Pharmacol. 367, 562-571.

(34) Witte I., Kreienkamp H.-J., Gewecke, M. and Roeder, T. (2002) Putative histamine-gated chloride channel subunits of the insect visual system and thoracic ganglion. J. Neurochem. 83, 504-14.

(35) Windhorst, S., Frank, E., Georgieva, D.N., Genov, N., Buck, F., Borowski, P. and Weber, W. (2002) The major extracellular protease of the nosocomial pathogen Stenotrophomonas maltophilia: Characterization of the protein and molecular cloning of the gene. J. Biol. Chem. 277, 11042-11049.

(36) Wittenberger, T., Hellebrand, S., Munck, A., Kreienkamp, H.-J., Schaller, H.C. and Hampe, W. (2002) GPR99, a new G protein-coupled receptor with highest homology to a new subgroup of nucleotide receptors. BMC Genomics 3, 17.

Theses

Diploma

Henkel, Anette (2003) Identifizierung der ER-Signalsequenzen der Süß-Geschmacksrezeptoren rT1R1, rT1R2 und rT1R3 durch heterologe Expression ihrer extrazellulären Domänen. Universität Hamburg.

Meyer, Frauke (2000). Funktionelle Charakterisierung des *cis*-aktiven dendritischen Transportelements neuronaler MAP2-mRNA-Moleküle, Universität Hamburg.

Dissertations

Burhenne, Nicole (2003). Protein-Protein-Wechselwirkungen innerhalb der T1R-Familie der Umami- und Süßrezeptoren aus Rattus norvegicus (BERKENHOUT, 1769). Universität Hamburg.

Mameza, Marie Germaine (2003). Wechselwirkungen postsynaptischer Proteine in Mensch und Ratte. Universität Hamburg.

Segger-Junius, Mailin (2003).Charakterisierung der dendritischen Lokalisierung der shank1 mRNA. Universität Oldenburg.

Soltau, Michaela (2003). Untersuchungen zur Funktion des humanen Proteins shank1 in der Synaptogenese von Säugern. Universität Hamburg.

Walden, Martina (2002). Identifizierung und Charakterisierung fötaler RNA-Bindeproteine aus dem Rattengehirn. Universität Hamburg.

Wege, Konstanze (2003). Charakterisierung des MAP2-mRNA-Bindungsproteins MARTA1 aus dem Gehirn von *Rattus norvegicus*. Universität Hamburg.

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Neuronal cell fate specification

Ingolf Bach

A fundamental question of biology is how protein complexes consisting of multiple proteins regulate basic biological processes such as embryogenesis and, when disturbed, cause cancer. Our laboratory investigates the molecular mechanisms that underlie neuronal cell fate specification events during embryogenesis. As a model system we use the protein network around LIM homeodomain transcription factors (LIM-HD) (Fig. 1) and its regulation, applying molecular, biochemical and genetic methods.

Gene expression is controlled by transcription factors which are often part of larger protein networks. In recent years it has been discovered that the interactions of distinct cofactor complexes with transcription factors are decisive determinants for the regulation of gene expression. The LIM domain, a cysteine-rich zinc-coordinating motif, mediates protein-protein interactions and was originally discovered in LIM homeodomain transcription factors (LIM-HD). Other LIM domain-containing factors, referred to as LIM-only (LMO) proteins, are composed almost in their entirety of two tandem LIM domains. Both LIM-HD and LMO proteins are important developmental regulators critically involved in embryogenesis, and LMO proteins have additionally been implicated in oncogenesis.

The LIM homeodomain (LIM-HD) class of transcription factors has been shown in numerous examples to specify cell lineages and regulate neuronal differentiation and brain morphogenesis during the development of many species from *C. elegans* to humans. In particular, the development of motor- and interneurons has been shown to be dependent on the activity of various LIM-HD proteins. Furthermore, specific LIM-HD proteins are essential for the formation of many other neuronal and non-neuronal structures such as fore-, mid- and hindbrain, anterior pituitary and eye. Recent work indicates that LIM-HD transcription factors are present in cells as complexes consisting of multiple proteins and that their biological activity is regulated by LIM domain-associated cofactors CLIM and RLIM (Fig.1).

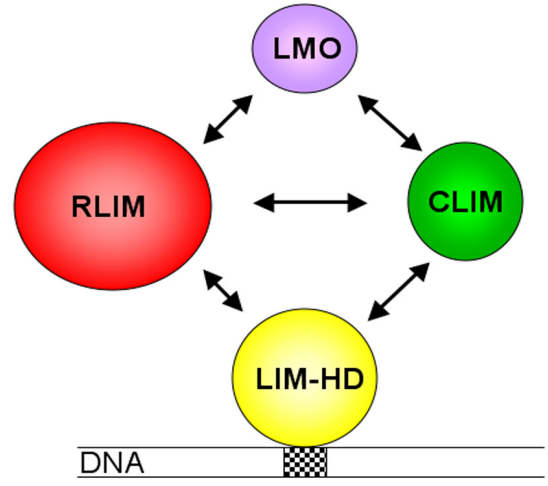

Figure 1: Protein network around LIM homeodomain transcription factors (LIM-HD). Cofactors of LIM proteins (CLIM), RING finger LIM interacting protein (RLIM) and LIM-only proteins (LMO) are shown. Black arrows indicate protein-protein interactions.

1. Regulation of developmental LIM-HD activity by LIM domain-associated CLIM cofactors

The CLIM (cofactor of LIM-HD proteins, also known as NLI, Ldb or Chip) cofactor family, consisting of CLIM1 and CLIM2, has been isolated by virtue of its ability to interact with the LIM domains of LIM-HD and LMO proteins. We and others have previously shown that CLIM and LIM-HD proteins associate *in vivo* with high affinity and form protein complexes on DNA. These protein interactions are required for synergistic gene activation events mediated by LIM-HD proteins *in vitro*. Previous genetic and biochemical results place the CLIM cofactor family in an essential position for controlling the biological activity of some LIM-HD proteins. We have generated a dominant-negative CLIM (DN-CLIM) molecule that upon overexpression can compete with wild-type CLIM for binding to LIM domains. To investigate if CLIM cofactors are important for the biological activity of many or all LIM-HD proteins we have ectopically overexpressed the DN-CLIM molecule early during zebrafish development via mRNA injections. Indeed, overexpression of DN-CLIM inhibited development of the eyes as well as the midbrain/hindbrain boundary, whereas development of the otic vesicle and other neuronal structures was not affected. We have identified additional functions of CLIM cofactors for the axonal outgrowth of specific neurons. The overexpression of DN-CLIM inhibited the development of peripheral but not central axonal projections in trigeminal and Rohon Beard neurons and motor neuron subtypes (Becker et al., 2002). These phenotypes parallel phenotypes obtained in specific LIM-HD knock out mice, indicating that CLIM cofactors are indeed important for many if not all LIM-HD proteins. Furthermore, the expression of the LIM-HD protein Isl1 in specific neurons that normally do not express this protein was induced upon DN-CLIM overexpression. Since this induction was detected in areas of low Isl1 mRNA synthesis, these results indicate that the association of CLIM proteins with LIM domains stabilizes LIM-HD factors (Becker et al., 2002), as is also the case for LMO proteins (Ostendorff et al., 2002; see below). Furthermore, we have demonstrated that CLIM cofactors are involved in the transcriptional activation of the cell adhesion molecule F3/contactin in a specific neuronal subpopulation of cells during zebrafish development (Gimnopoulos et al., 2002).

2. Role of the ubiquitin ligase RLIM in the LIM-HD protein network

Cellular proteins targeted for degradation are ubiquitinated by a cascade of enzymes involving ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). By bridging substrate proteins and E2 enzymes, the E3 enzymes are responsible for substrate specificity of the ubiquitination reaction. In general, poly-ubiquitinated proteins are recognized by the 26S proteasome and rapidly degraded.

RLIM (RING finger LIM domain-binding protein) is a LIM domain-interacting protein that can associate with many LIM-HD and LMO proteins. Besides LIM domains, RLIM is able to interact with CLIM cofactors and with members of the histone deacetylase corepressor (HDAC) complex. Our previous results indicated that RLIM functions as a negative regulator of LIM-HD activity. Prompted by the finding that E3 enzymes often contain a RING finger motif, we have identified RLIM as a ubiquitin ligase, able to ubiquitinate itself, CLIM and LMO proteins, but not LIM-HD factors *in vitro* (Fig. 2). We have demonstrated that RLIM is also able to poly-

ubiquitinate CLIM cofactors which are present in a LIM-HD/CLIM complex. RLIM-mediated LMO ubiquitination can be inhibited by addition of CLIM cofactors, showing a competition of RLIM and CLIM at the level of binding to LIM domains. In cotransfection experiments RLIM was able to induce degradation of CLIM. This proteolytic activity was inhibited by mutations in the RING finger of RLIM or by addition of specific inhibitors of the 26S proteasome. Although CLIM proteins bind with much higher affinity to LIM domains than RLIM *in vitro*, we demonstrated in chromatin immunoprecipitation experiments that RLIM was nevertheless able to induce a change in cofactors on LIM-HD proteins bound to DNA in transfected cells. This activity was dependent on the presence of a functional RING finger on RLIM. Since this RING finger is essential for CLIM ubiquitination and degradation, these experiments directly connect cofactor exchange on LIM-HD proteins with the ubiquitin-protein ligase activity of RLIM. Our data place RLIM in a central position for the developmental control of cellular CLIM cofactor levels and provide a mechanistic basis for a cofactor exchange on DNA-bound transcription factors (Fig. 2). Thus, RLIM is a ubiquitin ligase, regulating cellular cofactor abundance and the dynamics of nuclear multiprotein complexes (Ostendorff et al., 2002). The further ability of RLIM to ubiquitinate LMO oncoproteins argues for an additional role of RLIM in tumor formation.

Multiple substrate proteins have been identified for several ubiquitin ligases (Bach and Ostendorff, 2003). Since RLIM can interact with members of the HDAC corepressor complex we have examined in a collaborative effort whether proteins in the HDAC complex may serve as substrates for RLIM. Indeed, we were able to show that RLIM mediated the polyubiquitination and degradation specifically of HDAC2 (Krämer et al., 2003). As changes in HDAC2 are correlated with the occurence of specific tumors, these results provide additional evidence that RLIM may be involved not only in the regulation of embryogenesis and differentiation but also in tumor development.

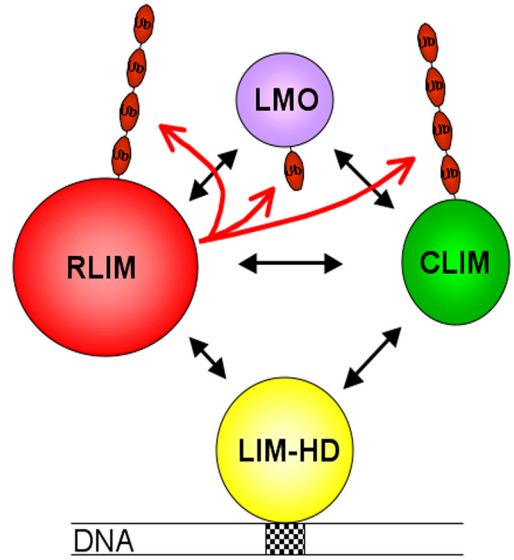

Figure 2: Model of the regulation of LIM-HD transcription factors by the cofactors CLIM and RLIM. Black arrows indicate protein-protein interactions. Red arrows indicate RING finger-mediated ubiquitination activity. By targeting LIM domain-associated CLIM cofactors for degradation, LIM-HD transcription factors can interact with other protein partners, e.g. RLIM, leading to a change in cofactor occupation.

Using ectopic overexpression of epitope-tagged proteins early during zebrafish development via mRNA injections, we recently developed a novel method that allows the comparison of protein stabilities between proteins (Becker et al., 2003). We will use this method in the future to identify and map specific instability domains on proteins.

Our results, taken together with the results from other laboratories, indicate that proteins participating in the LIM protein network play essential functions in cell fate specification events and neuronal differentiation. Furthermore, many of these proteins have been associated with pathological processes such as oncogenesis leading to human disease. Undoubtedly, the further deciphering of components of the protein network around LIM domains and the involved up- and downstream regulatory pathways will continue to illuminate new and exciting basic biological mechanisms.

Support

The work in our laboratory was supported by the Deutsche Forschungsgemeinschaft (grants BA1842/2-2, BA1842/3-2/3, BA1842/4-1, SFB444 and GRK255), the European Molecular Biology Organization, the Boehringer Ingelheim Fonds and the Alexander von Humboldt Foundation.

Publications

(1) Ostendorff, H.P.*, Peirano, R.I.*, Peters, M.A., Schlüter, A., Bossenz, M., Scheffner, M. & Bach, I. (2002). Ubiquitination-dependent cofactor exchange on LIM homeodomain transcription factors. *Nature* **416**, 99-103.

(2) Becker, T., Ostendorff, H.P., Bossenz, M., Schlüter, A., Becker, C.G., Peirano, R.I. & Bach, I. (2002). Multiple functions of LIM domain-binding CLIM/NLI/Ldb cofactors during zebrafish embryogenesis. *Mech. Dev*. **117**, 75-85.

(3) Ostendorff, H.P. & Bach, I. (2002). Roles of ubiquitin in nuclear function. *B.I.F.Futura* **17**, 207-215.

(4) Gimnopoulos, D., Becker, C.G., Ostendorff, H.P., Bach, I., Schachner, M. & Becker, T. (2002). Expression of the zebrafish cell recognition molecule F3/F11/contactin in a subset of differentiating neurons is regulated by cofactors associated with LIM domains. *Mech. Dev*. **119S**, S135-S141.

(5) Bach, I. & Ostendorff, H.P. (2003). Orchestrating nuclear functions: ubiquitin sets the rhythm. *Trends Biochem. Sci.* **28**, 189-195.

(6) Krämer, O.H., Zhu, P., Ostendorff, H.P., Golebiewski, M., Tiefenbach, J., Peters, M.A., Brill, B., Groner, B., Bach, I., Heinzel, T. & Göttlicher, M. (2003). The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2. *EMBO J.* **22**, 3411-3420.

(7) Becker, T.*, Bossenz, M.*, Tursun, B., Schlüter, A., Peters, M.A., Becker, C.G., Ostendorff, H.P. & Bach, I. (2003). Comparing protein stabilities during zebrafish embryogenesis. *Methods Cell Sci.* **25**, 85-89.

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Thesis

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Neuronal Protein Degradation

Thorsten Hoppe

Protein degradation by the ubiquitin/proteasome system is prevocal for cellular regulation pathways, cell cycle progression, signal transduction and development. Substrates modified by multiubiquitin chains are usually marked for proteolysis by the 26S proteasome, a multicatalytic protease complex. Ubiquitylation of proteins requires a cascade of enzymes. Ubiquitin-activating enzyme, E1, hydrolyzes ATP and forms a high-energy thioester bond between an internal cysteine residue and the C-terminus of ubiquitin. Activated ubiquitin is then passed on to ubiquitin-conjugating enzymes, E2s, which form similar thioester-linked complexes with ubiquitin. Finally, ubiquitin is covalently attached to the substrate protein by ubiquitin-protein ligases, E3s, which often interact with the substrate directly. Recently, the yeast protein UFD2 has been described as an additional conjugation factor, E4, which binds to the ubiquitin moieties of preformed conjugates and catalyzes multiubiquitin chain assembly in conjunction with E1, E2, and E3. E4-mediated multiubiquitylation is needed for proteasomal targeting and subsequent proteolysis of specific model substrates.

The human CHIP protein (Carboxyl-terminus of Hsc70 interacting protein) also displays E4 function, since it positively regulates the ubiquitylation activity of the E3 enzyme Parkin. Therefore it might play a role as an E4 enzyme in the development of autosomal-recessive juvenile Parkinsonism (AR-JP), one of the most common forms of Parkinson's disease. Additionally, CHIP shows an important role in the regulation of the microtubule associated protein tau and seems to be involved in the pathogenesis of tauopathies. Mammalian UFD2a, an ortholog of the yeast E4 enzyme UFD2, has been identified as a rate-limiting factor in the degradation of pathological forms of ataxin-3, which are responsible for spinocerebellar ataxia type 3 (SCA3, also known as Machado-Joseph disease). Furthermore, the aberrant expression of a mutant Ufd2/D4Cole1e fusion protein has been implicated in slow wallerian degeneration mice.

Both CHIP and UFD2 contain a U-box domain at the C-terminus which mediates binding and ubiquitylation of substrate proteins. In addition to its C-terminal U-box, CHIP contains three tandem tetratricopeptide repeat (TPR) motifs at the N-terminus. These TPR motifs bind to the chaperones Hsp70 and Hsp90, thereby mediating the co-chaperone activity of CHIP. Thus, CHIP provides a direct link between the chaperone and proteasome systems, and is postulated to assist in regulating the cellular balance between folding and degradation. This hypothesis is supported by the observation that *in vivo* overexpressed CHIP is found in activation- and folding-competent chaperone complexes with glucocorticoid receptor (GR), cystic fibrosis transmembrane conductance regulator (CFTR) and ErbB2, leading to ubiquitylation of these substrates and acceleration of their degradation through the 26S proteasome. Furthermore, heat-denatured polypeptides might also be substrates for CHIP-dependent ubiquitylation.

Our research focuses primarily on functional aspects of E4 enzymes. One of them is a *C. elegans* homolog of yeast UFD2. The other protein we are studying is the *C. elegans* homolog of the human CHIP protein, CHN-1. The multiubiquitylation activities of these E4 enzymes may serve

as an accessory option to regulate proteolysis by ubiquitin chain elongation. We are interested in the identification of novel genetical and physical interaction partners and substrates. The detailed analysis of binding partners and of substrate-ubiquitylation *in vivo* and *in vitro* will help decipher the exact mechanism how this novel enzymatic activity, ubiquitin chain elongation, occurs, how it regulates specific protein turnover throughout development and the specific role in neurodegeneration.

Another major aspect will be the identification of new components of the ubiquitin/proteasome system involved in neuron-specific protein degradation. Therefore we will establish a tissue specific *in vivo* degradation assay in a living organism. The isolation of new genes implicated in neuron-specific protein degradation and their genetic and biochemical characterization will open a completely new avenue of research. Moreover, this might give further insights into the connection between the ubiquitin/proteasome system and neurodegenerative diseases like Parkinson's and Alzheimer's disease.

Most human disease genes expressed throughout the nervous system are not conserved in yeast and thus can not be studied in this simple, monocellular organism. Consequently it is necessary to address these questions in a higher eukaryotic system. Since many aspects of studying selective protein degradation in higher eukaryotes might be very complex, we have chosen the small nematode worm *Caenorhabditis elegans* as a model organism that facilitates true genetic analysis. This multicellular organism has a short life cycle, its cultivation is easy and cost effective and it is small enough to be handled in large numbers. The nervous system consists of 302 neurons comprising 118 types that interconnect in a reproducible manner. Therefore our choice of *C. elegans* as an experimental organism is based on its multicellular organisation and its tractability for genetic analysis.

Support

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Publications

(1) Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H.D., Mayer, T.U. and Jentsch, S. (1999). A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell 96*, 635-644.

(2) Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H.D. and Jentsch, S. (2000). Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. *Cell 102*, 577-586.

(3) Rape, M., Hoppe, T., Gorr, I., Kalocay, M., Richly, H. and Jentsch, S. (2001). Mobilization of processed, membrane-tethered SPT23 transcription factor by $CDC48^{UFD1/NPL4}$, a ubiquitin-selective chaperone. *Cell 107*, 667-677.

(4) Hoppe, T., Rape, M. and Jentsch, S. (2001). Membrane-bound transcription factors: regulated release by RIP or RUP. *Curr. Opin. Cell Biol. 13*, 344-348.

(5) Hertweck, M., Hoppe, T. and Baumeister, R. (2003). C. elegans, a model for aging with high-throughput capacity. Exp Gerontol. 38, 345-346.

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Sorting, Transport and Synapse Formation

Matthias Kneussel

The research of our group is concerned with sorting and transport mechanisms of neurotransmitter receptors toward and/or from dendrites and postsynaptic sites, respectively. Furthermore, we study the anchoring of receptors at developing postsynaptic specializations. We apply proteomic, biochemical and imaging approaches on living neurons to investigate motor protein-dependent transport reactions, which underlie synapse formation and plasticity. Additional yeast two-Hybrid approaches should identify new protein-protein interactions in the transport of synaptic components. These aspects are expanded by the generation of transgenic mouse lines expressing GFP-fusion proteins of pre- and postsynaptic components to visualize synapse remodeling upon network stimulation.

1. Sorting and transport of neurotransmitter receptors

Axons and dendrites perform different functions: the dendritic surface receives and processes information while the axonal surface is specialized for the rapid transmission of electrical impulses. Consistent with these different physiological properties of both domains, components of the presynaptic active zone and the postsynaptic density are preferentially distributed either to the axonal or somatodendritic compartment, respectively. Within neuronal dendrites, neurotransmitter receptors have to pass another step of sorting, which directs individual receptors of either the excitatory or inhibitory type toward a spine or shaft synapse. To understand the underlying mechanisms of selective sorting of neurotransmitter receptors to distinct postsynaptic specializations in neuronal dendrites, two possible mechanisms will have to be considered: i.) selective targeting and ii.) unspecific targeting and/or selective removal. The selectivity of targeting to either the spine or shaft synapse could be achieved by the segregation of receptors into different carriers that are delivered only to the plasma membrane of the appropriate postsynaptic domain. Alternatively, receptors could be equally delivered to the surface membrane at „a priori“ unspecific locations and subsequently be retained only in the appropriate domain; thus selectivity, would be achieved downstream of transport. In general, a number of different mechanisms should be considered to act in combination in order to achieve postsynaptic delivery of individual neurotransmitter receptors toward either an excitatory or inhibitory synapse, located either on spines or shafts: (1) receptors might be sorted to different post-Golgi carriers already at the level of the trans-Golgi network (TGN); (2) transport packages might be directed to different subdomains within the dendrite; (3) the fusion between individual transport vesicles and the plasma membrane may be selectively regulated, as for instance by the nature of membrane lipids; (4) receptors might enter the surface membrane at unspecific positions and then subsequently reach the synapse via lateral diffusion; (5) diffusion barriers within the plasma membrane might hinder individual receptors to enter or leave specific compartments; and (6) mechanisms which differentially regulate receptor endocytosis and receptor turnover might contribute to receptor densities at a given time and location.

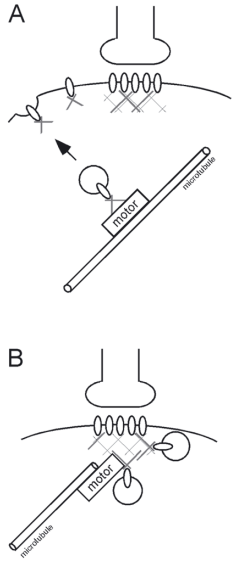

Figure 1. Schematic representation of possible alternative mechanisms for the postsynaptic delivery of neurotransmitter receptors. (A) Transport packages deliver receptors to extrasynaptic membrane locations. Receptors travel within the plane of the plasma membrane toward axo-dendritic contact sites, either via active mechanisms or lateral diffusion. (B) Transport packages dock at postsynaptic specializations and deliver receptors directly toward the appropriate synapse.

2. The dynein motor complex in gephyrin transport

The scaffold protein gephyrin is a subsynaptic component of the postsynaptic density (PSD) at inhibitory synapses. Gephyrin function is critical for the synaptic localization of glycine receptors and individual GABA-A receptors. We identified the dynein light chains Dlc-1 and Dlc-2, components of the dynein motor complex, as direct interaction partners of gephyrin polypeptides. Dynein light chains are enriched at inhibitory postsynaptic sites and are suggested to particpate in cargo binding of gephyrin transport packages. The neuronal expression of gephyrin deletion mutants, which lack the Dlc-binding site, leads to normal synaptic localization of postsynaptic gephyrin. Our data suggest, that the dynein motor participates in retrograde transport reactions of transport packages containing gephyrin and/or associated components.

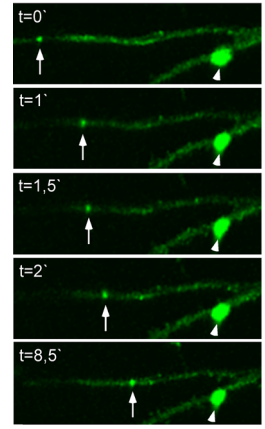

Figure 2. Time-lapse imaging of a green fluorescent protein (GFP)-fused synaptic component, expressed in cultured hippocampal neurons. Individual transport packages are highly mobile within neuronal dendrites.

3. Anchoring of GABA-A receptors at postsynaptic membrane specializations

Neurotransmitter receptors, which arrive at the neuronal surface are subject to lateral diffusion and/or intra-membrane transport across the plane of the cellular plasma membrane. Underlying synapse formation, receptor polypeptides need to concentrate at sites of axo-dendritic contact, which release the appropriate neurotransmitter. This process of receptor clustering is achieved by direct or indirect association with cytoskeletal components. Clustering processes of inhibitory GABA-A receptors are currently barely understood. Individual subtypes of GABA-A receptors depend on the scaffold protein gephyrin, which primarily anchors inhibitory glycine receptors at postsynaptic sites, since gephyrin knockout mice also display a partial reduction of certain GABA-A receptor clusters. To investigate the development of GABAergic postsynaptic specializations, we search for additional protein-protein interactions, which participate in receptor anchoring. Our data suggest that different proteins participate in GABA-A receptor clustering processes.

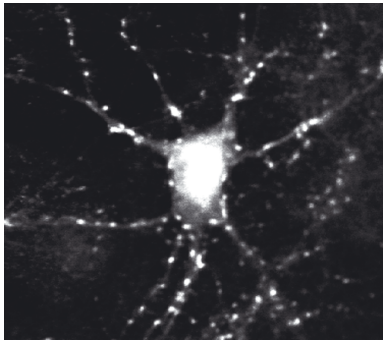

Figure 3. Expression of a green-fluorescent fusion protein as a marker for inhibitory post-synaptic membrane specializations in hippocampal neurons cultured for 15 days *in vitro*

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft (grants KN556/1-1; KN556/1-2; SFB444/B7)

Publications

(1) Kneussel, M., Brandstätter, J.H., Laube, B., Stahl, S., Müller, U. and Betz, H. (1999). Loss of postsynaptic GABA-A receptor clustering in gephyrin-deficient mice. J. Neurosci. 19, 9289-9297.

(2) Kneussel, M., Haverkamp, S., Fuhrmann, J.C., Wang, H., Wässle, H. and Betz, H. (2000). The GABA-A receptor-associated protein GABARAP interacts with gephyrin but is not involved in receptor anchoring at the synapse. Proc. Natl. Acad. Sci. USA 97, 8594-8599.

(3) Bavro, V.N., Sola, M., Bracher, A., Kneussel, M., Betz, H. and Weissenhorn, W. (2002). Crystal structure of the GABA-A receptor-associated protein GABARAP. EMBO Rep. 3, 183-189.

(4) Fuhrmann, J.C., El Far, O., Sheng, M., Kirsch, J., Triller, A., Betz, H. and Kneussel, M. (2002) Gephyrin interacts with Dynein light chains 1 and 2, components of Dynein and Myosin Va Motor Protein Complexes. J. Neurosci, 22, 5393-5402.

(5) Lee, H.J., Adham, I.M., Schwarz, G., Kneussel, M., Sass, J.O., Engel, W. and Reiss, J. (2002). Molybdenum cofactor-deficient mice resemble the phenotype of human patients. Hum. Mol. Genet. 11, 3309-3317.

(6) Grosskreutz, Y., Betz, H. and Kneussel, M. (2003). Rescue of molybdenum cofactor biosynthesis in gephyrin-deficient mice by a Cnx-1 transgene. Biochem. Biophys. Res. Commun. 301, 450-455.

Review Articles

(7) Kneussel, M. and Betz, H. (2000). Clustering of inhibitory neurotransmitter receptors at developing postsynaptic sites: the membrane activation model. Trends Neurosci. 23,429-435.

(8) Kneussel, M. (2002). Dynamic regulation of GABA-A receptors at synaptic sites. Brain Res. Rev. 39, 74-83.

Thesis

Habilitation

Kneussel, Matthias (2004). Molekulare Organisation von membranständigen und membran-assoziierten Proteinen zentraler Synapsen im Nervensystem von Säugern. JohannWolfgang Goethe-Universität Frankfurt/Main.

Award

Janssen-Cilag Preis der Deutschen Neurowissenschaftlichen Gesellschaft to Matthias Kneussel, Juni 2001.

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Molecular analysis of synaptic modulation: protein interactions of metabotropic receptors

Hans-Christian Kornau

Synapses are highly specialized structures fulfilling the complicated task of neuronal communication. Coordinated cascades of protein-protein interactions mediate transmission of electrical signals from the pre- to the postsynaptic cell via regulated release and detection of a soluble transmitter. The efficiency of synaptic transmission is frequently modulated by metabotropic pathways. To improve our understanding of synaptic physiology and cross-talk we are studying the physical interactions of synaptic G-protein-coupled receptors (GPCRs) and their functional implications.

$GABA_B$ receptors, the metabotropic receptors for the principal inhibitory neurotransmitter GABA, modulate transmission at both glutamatergic and GABAergic synapses in the central nervous system. Upon ligand binding these receptors activate trimeric $G_{i/o}$-proteins opening inwardly rectifying potassium channels and inhibiting voltage-dependent calcium channels as well as adenylate cyclase activity. $GABA_B$ receptors localized on axon terminals confer inhibitory effects on transmitter release, whereas postsynaptic receptors elicit hyperpolarization by an increase in membrane potassium conductance.

Our initial molecular analysis had revealed that the functional $GABA_B$ receptor consists of two subunits, $GABA_{B1}$ and $GABA_{B2}$, with seven transmembrane domains each. (Kuner et al. (1999) Science 283, 74-77). Subsequent studies addressed the functional role of the C-terminal intracellular domains of the receptor subunits in assembly, cell surface trafficking and G-protein coupling (1). We showed that the two proteins interact via at least two different sites, a C-terminal coiled-coil interaction and an additional interaction in the extracellular or transmembrane region of the two molecules. The coiled-coil interaction primarily regulates the surface trafficking of the $GABA_B$ receptor by masking an ER retention signal in $GABA_{B1}$. We found that the C-terminal sequences are not necessary for agonist-dependent G-protein coupling, but influence the constitutive activity of the receptor. Our current work aims at identifying proteins associated with the $GABA_B$ receptor complex using genetic and biochemical techniques to unravel the regulation of surface trafficking of the receptor, targeting to specific pre- and postsynaptic sites and the nature of receptor subtypes.

The $GABA_B$ receptor functions only in the heterodimeric configuration, most likely because the ligand-binding and the G-protein-coupling functions are separated onto the two subunits. However, several additional GPCRs that can function as monomers have recently been shown to be part of heteromeric complexes as well. Moreover, these complexes can consist of receptors for different ligands or even of receptors from different protein families, including ion channels, tyrosine kinases and GPCRs. Thus, a major focus of our work is to analyse interactions of integral membrane proteins. For this purpose we apply a β-lactamase based complementation system.

1. Novel candidate interactors of metabotropic receptors identified by genetic screens in yeast

Although several binding partners of the C-terminal domains of the $GABA_B$ receptor have been described, the tight regulation of cell surface trafficking, the presence of receptor subtypes in the mammalian brain as well as the pre- and postsynaptic localization of the receptor suggest additional interactions. We apply both classical yeast two-hybrid (Y2H) screening as well as the ras recruitment system (RRS) to identify interaction partners of the $GABA_B$ receptor complex and other metabotropic receptors.

Application of the RRS with the C-terminus of $GABA_{B1}$ as a bait identified the previously described interaction partners $GABA_{B2}$, 14-3-3 and ATF4 demonstrating the validity of the system. In addition, our screens yielded several novel cDNAs, whose specific binding to the bait was confirmed in subsequent co-transformations. These clones are currently analyzed by biochemical and functional assays.

We also applied the Y2H system using dopamine receptor baits. Screening with the third cytoplasmic loop of the dopamine D2 receptor detected the previously described interaction with spinophilin (Smith et al. (1999) J. Biol. Chem. 274, 19894-19900). However, using the C-terminus of Calcyon, a single transmembrane protein modulating dopamine D1 receptors, as a bait in the Y2H system identified a novel interaction with an uncharacterized protein containing several TPR repeats. We confirmed this interaction using glutathione-S-transferase (GST) pull-down assays.

To functionally analyse the identified interactions we currently focus on measuring cell surface trafficking, stability, internalization and G-protein coupling of the receptors in presence and absence of the candidate interaction partners in heterologous expression systems.

2. Tagging of metabotropic GABA receptors in mice

Alternative transcriptional start site selection in the $GABA_{B1}$ gene results in the variants $GABA_{B1(a)}$ and $GABA_{B1(b)}$, which differ in the presence of two sushi repeats at the aminoterminus of $GABA_{B1(a)}$. These extracellular protein interaction domains have previously been found in proteins of the complement system. The two variants show differential expression patterns and may form the basis for receptor subtypes based on differential localization to specific cellular microdomains.

To study the $GABA_B$ receptor complexes we generate transgenic mice expressing tagged $GABA_{B1}$ subunits. Using ET recombination we introduced DNA sequences encoding affinity tags into different sites of a bacterial artificial chromosome (BAC) encompassing the $GABA_{B1}$ gene. Transgenic mice carrying the modified BACs will express additional $GABA_{B1}$ subunits carrying tags for affinity purification either in both variants or specifically in the $GABA_{B1(a)}$ variant. We confirmed that the tagged receptors behave like wild-type receptors with respect to ligand-stimulated G-protein coupling upon expression in heterologous cells and established a protocol allowing efficient purification of the tagged receptors from these cells. Applying this protocol to brain protein samples of the transgenic mice should allow purification and subsequent mass spectrometry identification of proteins associated with $GABA_B$ receptor complexes.

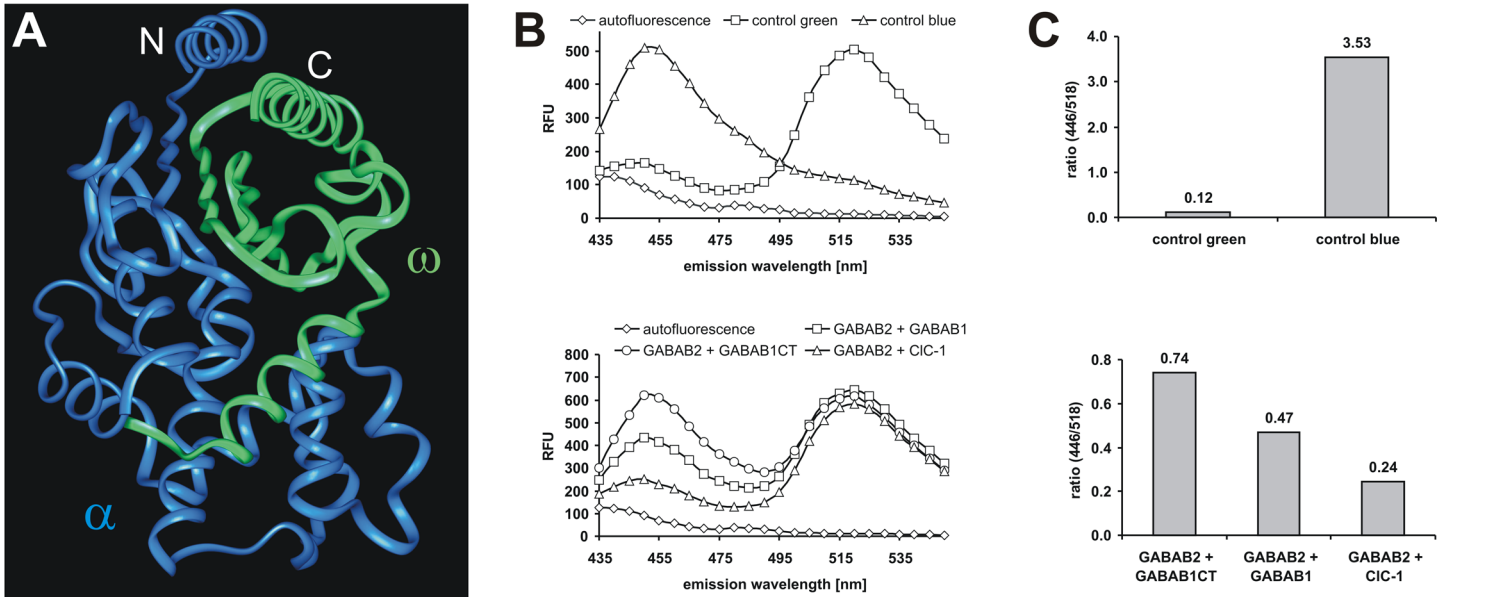


Figure 1. Monitoring membrane protein interactions using the b-lactamase complementation system. (A) Three dimensional structure of the b-lactamase enzyme. The N-terminal a and the C-terminal w fragment used for the construction of fusion proteins are depicted in blue and green, respectively. (B) Emission spectra of CCF2/AM loaded cells. b-lactamase activity is detected by an emission wavelength shift from green (518nm) to blue (446nm). Upper panel: comparison of FlpIn3T3 cells not loaded with CCF2/AM (autofluorescence), FlpIn3T3 parental cells loaded with CCF2/AM (control green) and CCF2/AM loaded FlpIn3T3 cells stably expressing enzymatically active b-lactamase (control blue). Lower panel: comparison of FlpIn3T3 cells stably expressing full-length $GABA_{B2}$ C-terminally fused to the w fragment of b-lactamase (GABAB2) infected with retroviral constructs encoding C-terminal a fragment fusions to the soluble C-terminal fragment of $GABA_{B1}$ (GABAB1CT), full length $GABA_{B1}$ (GABAB1), or the ClC-1 chloride channel (ClC-1). Two days after infection cells were loaded with CCF2/AM and emission spectra were recorded using a Spectramax Gemini fluorimeter at an excitation wavelength of 409nm. RFU = relative fluorescence units. (C) Ratiometric quantification of the fluorescence emission spectra in (B). After subtraction of autofluorescence the fluorescence intensities measured at wavelengths 446nm (blue fluorescence) and 518nm (green fluorescence) were used to calculate the ratio of blue to green fluorescence (ratio (446/518)).

3. β-lactamase complementation assay

The b-lactamase complementation system is based on the prokaryotic enzyme b-lactamase. This 29-kDa protein can be split in two enzymatically inactive fragments (designated a and w, Fig.1A) that can reconstitute a functional b-lactamase enzyme when they are expressed as fusion partners of a pair of interacting proteins (Wehrman et al.

(2002) Proc. Natl. Acad. Sci. 99, 3469-3474). β-lactamase activity can be monitored either *in vitro* (cell lysates) by using the colorimetric substrate Nitrocefin or *in vivo* (living cells) by applying the membrane-permeant fluorescent substrate CCF2/AM.

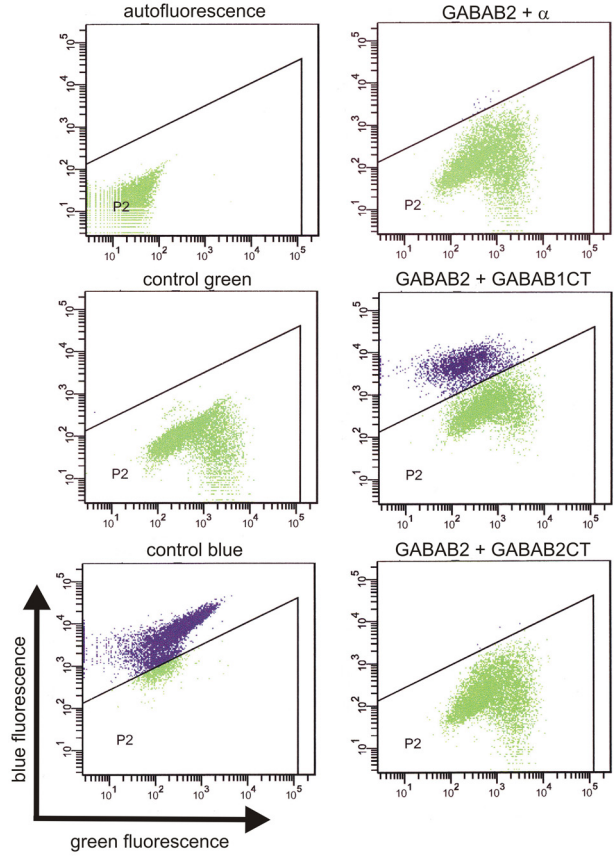
Significant advantages of the β-lactamase system are the monomeric structure of the β-lactamase enzyme, the lack of endogenous β-lactamase activity in mammalian cells, the enzymatic amplification of the read-out signal and the existence of a membrane-permeant fluorescent substrate (CCF2/AM) being suitable for fluorimetric measurements, fluorescence microscopy and fluorescence-activated cell sorting (FACS). The CCF2/AM substrate offers the possibility to perform ratiometric fluorescence measurements, giving rise to read-outs that are independent of cell number, size and cell-loading intensity. Furthermore, the β-lactamase complementation system is not restricted to a special cellular compartment as the nucleus or to soluble fusion proteins, i.e. also full-length membrane proteins can be studied.

To set up the β-lactamase complementation system in our laboratory, we first analyzed the well-known constitutive coiled-coil interaction between the intracellular C-terminal parts of $GABA_{B1}$ and $GABA_{B2}$. When fused to the α and the ω fragment of β-lactamase, respectively, co-expression of these soluble proteins resulted in a readily detectable, specific β-lactamase activity. These first experiments also revealed a dependency of the β-lactamase signal generated by the interacting fusion proteins from the relative orientation of the fusion partners. Such steric constraints are also inherent to other fusion protein-based assay systems as Y2H or fluorescence resonance energy transfer assays.

In further experiments we took advantage of the fact that not only constitutive interactions but also transient and inducible interactions can be studied using the β-lactamase system. The membrane stability of the $GABA_{B1}$-$GABA_{B2}$ heterodimer is markedly enhanced by PKA-mediated phosphorylation of a serine residue (S892) in the $GABA_{B2}$ C-terminal region (Couve et al. (2002) Nature Neurosci. 5, 415-424). Using the β-lactamase system, we were able to demonstrate a clearly phosphorylation-dependent interaction of a 20 amino acid fragment of the $GABA_{B2}$ C-terminus with a candidate binding partner that was proposed based on functional considerations and sequence homologies. These experiments serve as a starting point to reveal a possible role of this interaction in phosphorylation-mediated membrane stabilization of the $GABA_{B1}$-$GABA_{B2}$ heterodimer.

A major goal of our experiments is to monitor direct interactions between integral membrane proteins. To investigate the feasibility of the β-lactamase system regarding this demand, we analyzed the β-lactamase activity generated by combinations of the full-length $GABA_{B2}$ protein fused to the ω fragment with different interacting and non-interacting proteins fused to the α fragment. Initial co-transfection experiments indicated that application of the assay with respect to membrane protein interactions required a moderate expression of the interaction partners. Therefore, we generated a FlpIn3T3 cell line stably expressing low levels of the full-length $GABA_{B2}$-ω protein. These cells were infected with retroviral constructs encoding $GABA_{B1}$-α or the chloride channel ClC-1-α (kindly provided by Thomas Jentsch) as a negative control. Indeed, the specific $GABA_{B1}$-$GABA_{B2}$ interaction resulted in an increase in β-lactamase activity as compared to the control (Fig. 1). However, it appears that for the analysis of interactions between transmembrane proteins an increased background signal (see $GABA_{B2}$ + ClC-1 over control green in Fig. 1) most likely

Figure 2. FACS analysis of b-lactamase activity in FlpIn3T3 cells. FlpIn3T3 cells stably expressing a $GABA_{B2}$-w fusion protein (GABAB2) were infected with retroviral constructs encoding either the isolated a fragment (a) or N-terminal fusions of the a fragment to the soluble C-terminal domains of $GABA_{B1}$ (GABAB1CT) and $GABA_{B2}$ (GABAB2CT). Two days after infection, the cells were loaded with CCF2/AM and analysed using a FACS Aria (Becton Dickinson, San Jose, CA) equipped with excitation at 407nm and emission at 530/30nm (green fluorescence) and 450/40nm (blue fluorescence). For comparison, control FlpIn3T3 cells either expressing no b-lactamase activity (control green) or stably expressing b-lactamase (control blue) as well as FlpIn3T3 cells not loaded with CCF2/AM (autofluorescence) were analysed.



arising from unspecific aggregation of the fusion proteins has to be taken into account. This background was not observed when non-interacting soluble α fragment fusion proteins were co-expressed with $GABA_{B2}$-ω (data not shown). Moreover, the signal resulting from the interaction of $GABA_{B2}$ with the soluble $GABA_{B1}$CT protein clearly exceeded the signal resulting from the interaction of $GABA_{B2}$ with full-length $GABA_{B1}$ (Fig. 1). Nevertheless, our data show that interactions of two integral membrane proteins can be monitored using β-lactamase complementation.

We aim at extending the β-lactamase complementation system into a mammalian two-hybrid screening system. For this purpose we will infect cell lines stably expressing the bait protein with a retroviral cDNA expression library. Separation of cells expressing interaction partners will be achieved by FACS of CCF2/AM-loaded cells. We are currently establishing this sorting process using the FlpIn3T3/$GABA_{B2}$-ω cell line infected with viruses expressing α fragment fusions of various cytosolic and membrane-spanning control proteins. Preliminary results show that the blue fluorescence of cells harbouring β-lactamase activity based on a specific interaction with $GABA_{B2}$ allows their FACS separation from cells co-expressing a non-interacting protein (Fig. 2).

Support

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Publication

(1) Grünewald, S., Schupp, B.J., Ikeda, S.R., Kuner, R., Steigerwald, F., Kornau, H.-C. and Köhr, G. (2002). Importance of the gamma-aminobutyric acid (B) receptor C-termini for G-protein coupling. Mol. Pharmacol. 61, 1070-1080.

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Development of the peripheral nervous system

Dieter Riethmacher

The group's main focus in the past years was the analysis of differentiation processes in the peripheral nervous system. Inactivation of the tyrosinekinase-receptor erbB3 and the transcription factor sox10 in the mouse has highlighted their importance for glial development. Both genes are also expressed in oligodendrocytes, the myelinating glia of the central nervous system, and consequentially we extended our analysis on the development of these cells. This analysis led us also into the field of stem cell research, as we employed erbB3 and sox10 mutant embryonic and neural stem cells in our studies.

Another topic of our research is the generation of a universal mouse model for cell ablation. Loss of cells has a great impact on the whole organism not only during development but also in pathogenesis and homeostasis. Therefore the specific ablation of cells in an intact organism will make it possible to model diseases and help to understand their pathogenesis and also to unravel functions of tissues or cell types during development and homeostasis. To this end we have generated a mouse line that enables us to efficiently ablate any cell type *in vivo* after cre-mediated recombination by introducing a b-galactosidase(flox)-diphtheria toxin fragment A (lacZ(flox)-DT-A) cassette into the ubiquitously expressed ROSA26 locus. We have characterized the DT-A-line by mating it with different Cre-expressing lines.

1. Functions of Sox10 in oligodendrocytes

Sox10 expression in the oligodendrocyte lineage starts early in development and is maintained in mature oligendrocytes throughout adulthood. The analysis of embryonic development showed that surprisingly the oligodendrocyte lineage is generated normally as revealed by the presence of several markers. This is in contrast to the roles of sox10 in the PNS where gliogenesis is completely blocked. In late embryogenesis, oligodendrocyte precursors begin to terminally differentiate and to express myelin genes such as MBP, PLP and MAG. Sox10 mutant embryos showed a dramatic decrease in the number of cells expressing those late markers. Only very few (appr. 5%) cells can be found in mutants (Fig.1). As mutant embryos die latest at birth further development could not be analyzed and therefore neural stem cells were transplanted into the retinas of recipient animals to follow the fate of these cells in vivo. Under these conditions wildtype cells differentiate into myelin-forming oligodendrocytes while sox10 mutant cells failed to do so (Fig.1)

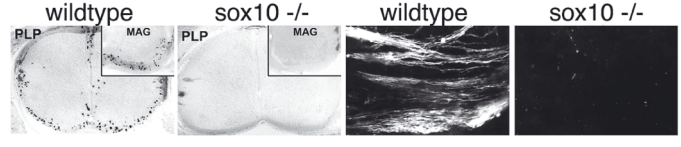

Figure 1. Left: In-situ-hybridisation with MAG- and PLP-specific probes on cross sections of E18.5 embryos demonstrating the dramatic decrease of MAG and PLP expressing cells in mutant embryos. Right: Immunolabelling of flat-mounted retinas with MAG-specific antibodies. Transplantation of wildtype neural stem cells results in differentiation of myelinating oligodendrocytes while sox10 deficient neural stem cells fail to express myelin genes.

This analysis showed that terminal differentiation of myelin-forming oligodendrocytes is strictly dependent on sox10 gene function *in-vivo*.

2. ErbB3 in oligodendrocytes; lessons from embryonic and neural stem cells

Unlike sox10 the erbB3 gene is not expressed in oligodendrocyte precursors and its expression begins just around birth in this lineage. Hence, it was not surprising that mutants do not display any obvious phenotype in oligodendrocytes at birth, the latest stage that can be analyzed due to the perinatal lethality. However, several observations suggested an important role for neuregulin-erbB-signalling in oligodendrocytes and therefore we decided to investigate possible functions of the erbB3 gene in oligodendrocytes by two alternative techniques. First we directed the differentiation of erbB3 deficient embryonic stem (ES) cells into neural cell types to analyse the development of oligodendrocytes in the absence of erbB3 *in-vitro*. Second, we grafted neural stem cells from spinal cords of erbB3 mutants into the retina of young mice to monitor oligodendrocyte differentiation and myelination *in-vivo*.

Neural differentiation of erbB3 deficient and control ES cells was induced and monitored at various stages of the differentiation protocol for the appearance of characteristic marker proteins. Varying amounts of neurons, astrocytes and oligodendrocytes (Fig. 2 a,d) could be identified with specific antibodies at the end of the differentiation protocol. In all these analyses we could not detect apparent differences in marker gene expression between erbB3 deficient and control cultures. Also quantification of the immunolabelling experiments resulted in similar numbers for all analysed markers in wild-type and mutant cultures, clearly demonstrating that erbB3 is dispensable for oligodendrocyte differentiation of ES cells *in-vitro*. NSC cultures were prepared from E12.5 erbB3 deficient and wild-type embryos and expanded in serum-free medium in the presence of EGF and bFGF. Differentiation was induced by growth factor withdrawal. No conspicuous difference regarding proliferation, morphology or marker gene appearance was observed between wild-type and mutant cultures in these experiments (Fig. 2 b,e), demonstrating that cultured erbB3 deficient NSCs are capable to differentiate into all three major cell types of the mammalian brain *in-vitro*.

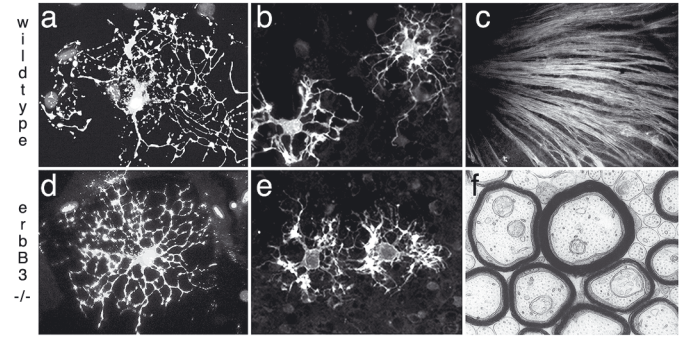

Figure 2. Immunofluorescence analysis of *in-vitro* differentiated embryonic stem cells with oligodendrocyte specific anti-GalC-antibodies in controls (a) and erbB3 mutant cells (d). Analysis of neural stem cell cultures with anti-MAG-specific antibodies after *in-vitro* differentiation of control (b) and erbB3 deficient cultures (e). Immunolabelling of flat-mounted retinas with MAG-specific antibodies following transplantation of erbB3 deficient neural stem cells (c). Myelin generated in the absence of erbB3 appears normal by electron microscopic analysis (f).

Transplantation of these neural stem cells into the retina of recipient mice revealed that MAG positive (Fig. 2 c) oligodendrocytes could be generated in the absence of erbB3. Combining the *in-vitro* and *in-vivo* data we have shown that erbB3 is dispensable during all phases of oligodendrocyte development. This is in clear contrast to the function of erbB3 in the PNS where it is absolutely required for Schwann cell development and myelin maintenance.

3. Lineage ablation in mice using DT-A

Abnormal cell loss is the common cause of a large number of developmental and degenerative diseases. To model such diseases in transgenic animals, we have developed a line of mice that allows the efficient ablation of virtually any cell type *in vivo*. We integrated our construct into the ubiquitously expressed ROSA26 locus by homologous recombination. In our construct the loxP-flanked lacZ ORF is inserted into the DT-A ORF after the ATG of DT-A thus allowing the expression of the toxic gene product after Cre-mediated excision of the lacZ gene. To verify the functionality of our construct we transiently transfected ES cell clones with plasmid vectors driving the expression of either EGFP, Cre-recombinase or both together. These experiments demonstrated that after Cre mediated removal of the lacZ-ORF diphtheria toxin becomes active and leads to cell death within 2-3 days after its expression.

To analyse specific deletion *in-vivo* we mated the DT-A-line with Nex-Cre animals that express Cre in pyramidal, postmitotic neurons of the cortical plate shortly after they are generated (E11.5). Mutant animals were born but died within the first 24 hours displaying a severe malformation of the cortex. TUNEL analysis revealed massive cell death in the cortices from E13.5 on confirming the kinetics for *in-vitro* cell ablation also *in-vivo*. Immunohistochemistry confirmed the elimination of Nex-(Cre)-expressing cells which was complete despite the permanent production of new nex-expressing cells (Fig. 3 a, d).

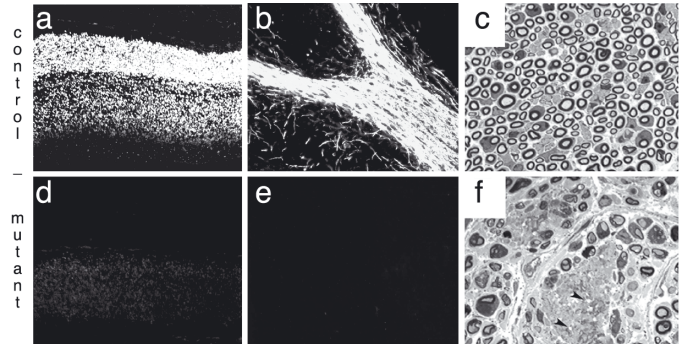

Figure 3. Analysis of cell ablation after Cre-mediated recombination. (a,d) Immunohistochemical analysis of E16.5 cortices with a Cre specific antibody in nex-cre/DT-A embryos. Note the almost complete absence of immunoreactivity in the mutant (d) compared to the distinct staining in the different layers from the control (a). Immunofluorescent analysis from P14 CNP-Cre/DT-A animals with MBP-specific antibodies showing the complete absence of oligodendrocytes in the cerebellum from mutants. Electron microscopy on sciatic nerves of P14 control (c) and mutant (f) animals showing the severe loss of myelin and myelinating Schwann cells.

CNP-Cre animals express Cre in oligodendrocytes and Schwann cells. When mated with the DT-A line mutant offspring was apparently normal regarding behaviour and early postnatal development. From P10 on they showed gait abnormalities and trembling and died within the next 4 days. The phenotypic behaviour reminded with known myelin mutants and is in good agreement with a defect in the oligodendrocyte lineage. In our immunohistochemical

analysis we were not able to detect oligodendrocytes or the precursors at any stage during late embryogenesis or postnatally (Fig. 3 b,e). While the oligodendrocyte lineage was completely eliminated the Schwann cell lineage was only partially ablated and consequently myelin could be found in peripheral nerves (Fig. 3 c,f). CNP expression levels are significantly lower in Schwann cells compared to oligodendrocytes and it is not present in the precursors. These differences can explain the partial ablation of the Schwann cell lineage compared to the complete ablation of oligodendrocytes.

Having demonstrated the functionality of our *in-vivo*-ablation system we are currently trying to combine it with stem cell technology. As the elimination of endogenous tissues is genetically controlled, exogenous tissues will not be affected by the ablation and therefore our model will offer new opportunities for stem cell research, as the regenerative potential of cells or tissues can be studied by transplantations into cell-ablated animals.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft (SFB444) and by the BMBF (NGFN).

Publications

(1) Stolt, C. C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U., and Wegner, M. (2002). Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes Dev 16, 165-70.

(2) Proikas-Cezanne, T., Stabel, S., and Riethmacher, D. (2002). Identification of protein tyrosine phosphatase 1B and casein as substrates for 124-v-Mos. BMC Biochem 3, 6.

(3) Schmucker, J., Ader, M., Brockschnieder, D., Brodarac, A., Bartsch, U. and D. Riethmacher (2003). ErbB3 is dispensable for oligodendrocyte development in-vitro and in-vivo. Glia 44, 67-75.

(4) Alvarez, Y., Alonso, M.T., Vendrell, V., Zelarayan, L. C., Chamero, P., Theil, T., Bosl, M. R., Kato, S., Maconochie, M., Riethmacher, D., and Schimmang, T. (2003). Requirements for FGF3 and FGF10 during inner ear formation. Development 130, 6329-6338

(5) Brockschnieder, D., Lappe-Siefke, C., Goebbels, S., Boesl, M. R., Nave, K. A. and Riethmacher, D. (2004). Cell ablation through diphtheria toxin fragment A (DT-A) after Cre mediated recombination. Mol Cell Biol (manuscript accepted for publication).

Theses

Dissertations

Brockschnieder, Damian (2003). Herstellung und Charakterisierung eines binär-genetischen Systems zur Zellablation in der Maus (Mus musculus) mittels Diphtherie Toxin. Universität Hamburg.

Miehe, Michaela (2003). ErbB3 defiziente Mäuse als System für die Analyse der Differenzierung und migration von Schwannzellen Diphtherie. Universität Hamburg.

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Mechanisms of Pancreas and Central Nervous System Development

Maike Sander

A cascade of molecular events leads to the differentiation of unspecialized progenitor cells into specialized cell types and the activation of cell-type-specific genes. Differentiated cell types are established and maintained by the correct temporal and spatial expression of transcription factors during development. Transcription factors have been shown to be involved in morphogenesis, and the establishment and maintenance of differentiated cell types. Our research aims to understand how certain transcription factors determine cell lineage decisions, specifically in the pancreas and central nervous system (CNS).

Interestingly, despite their different embryonic origin, pancreatic islet cells and neuronal cells in the CNS express remarkably similar sets of transcription factors during development. Previous research has identified that a number of key transcription factors regulate both neuronal differentiation and development of endocrine cells in the pancreas.

Given the similarities in the molecules expressed in pancreas and neural tube, it is our goal to define conserved developmental pathways utilized by both tissues. Specifically, we aim to identify other genes, and their function within the same developmental pathways as Nkx6.1. Our research employs biochemical methods, as well as animal models, using global and tissue-specific knockouts and over-expression studies.

1. Transcriptional Regulation of Pancreas Development

During pancreas development, a common endocrine precursor cell gives rise to four distinct endocrine cell types, each of which can be readily distinguished by the production of a characteristic hormone. Thus, the endocrine pancreas presents an ideal system to explore the roles of specific families of transcription factors in development.

The Nkx-family of transcription factors comprises a subfamily of the homeodomain transcription factors. Nkx genes are mammalian homologues of the Drosophila NK-transcription factors. All NK- and Nkx-transcription factors share a characteristic decapeptide sequence motif, which has been implicated in co-factor mediated transcriptional repression.

In previous studies, we had shown that Nkx6.1 is expressed in insulin-producing beta cells, as well as in their progenitors during embryogenesis. The absence of Nkx6.1 activity in mice leads to a defect in beta cell formation during development. Beta-cell numbers are reduced but not absent in Nkx6.1 mutant mice. This indicates that other genes can compensate for Nkx6.1 in beta-cell development. We found that the Nkx6.1 paralog, Nkx6.2, is also expressed in the pancreas. Expression of Nkx6.2 is only detected in the embryonic pancreas, but is absent from adult pancreas. A few cells coexpress Nkx6.1 and Nkx6.2 in the early pancreatic anlage, but later in pancreas development the expression domains become virtually exclusive. While Nkx6.1 is expressed in a subset of endocrine progenitors and in mature beta-cells, Nkx6.2 is expressed in glucagon-cells and exocrine cells. Interestingly, pancreatic expression of Nkx6.2 is strongly up-regulated in the absence of Nkx6.1, suggesting that Nkx6.1 represses Nkx6.2. To test if Nkx6.2 can

compensate for Nkx6.1, we analyzed pancreas development in Nkx6.2/Nkx6.1 double mutant embryos. Nkx6.2/Nkx6.1 double mutants show an almost complete loss of both insulin and glucagon cells. These data demonstrate redundant functions for Nkx6.1 and Nkx6.2 in the development of the endocrine lineage.

In another study, we explored the role of Sox transcription factors in pancreas development. Sox factors are a family of developmentally important transcription factors, which bind DNA through a highly conserved HMG-box. As all Sox factors bind the same target sequences, it is conceivable that different Sox factors compensate for each other in target gene activation. To begin to understand potential roles for Sox factors in the pancreas, we employed an expression analysis for Sox genes at different stages of pancreas development. Among the 20 mammalian Sox factors, we found expression of at least twelve members of this gene family in the pancreas. RNA in situ hybridization analyses revealed that some expression domains were unique for a particular Sox factor, while other Sox factors were expressed in overlapping domains. The expression of multiple members of the Sox gene family during pancreas development suggests that there could be functional redundancy. To begin to explore Sox gene function in the pancreas, we analyzed Sox10 and Sox8 mutant mice for defects in the development of the pancreas. While Sox8 mutant mice did not display any discernable defect in the pancreas, we found an absence of gial cells in the pancreas of Sox10 mutant mice. However, the perinatal lethality of Sox10 mutant mice precludes a further analysis of the relevance of glial cells for pancreatic functions, such as insulin secretion.

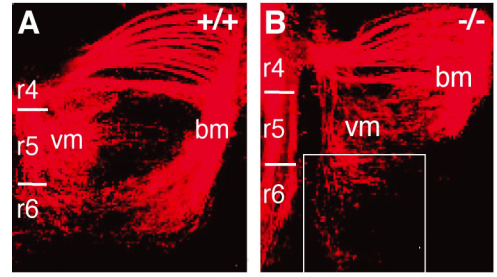

Figure 1. **Defects in branchiomotor neuron migration and axonal pathfinding in the hindbrain of *Nkx6.1* mutant embryos.** Application of DiI to the facial nerve in wild type (A) and *Nkx6.1* mutant embryos (B) at E12.5. While facial branchiomotor (bm) neurons are backlabeled along their entire migratory stream from r4 into dorsal r6 in wild type embryos (A), most facial bm neurons are clustered close to the ventral midline in r4 and few in the rostral third of r5 in *Nkx6.1* mutants (B). No difference in the location of facial visceromotor (vm) neurons is observed between wild type and *Nkx6.1* mutant embryos (A,B).

2. Nkx6.1 Function the Development of Spinal Cord Motor Neurons

Through an expression analysis of Nkx6.1, we found that Nkx6.1 is not only expressed in the pancreas, but also in the developing central nervous system (CNS). In the mouse embryo, neural expression of Nkx6.1 is first detected in the neural plate, and later in the neuronal progenitors throughout the ventral third of the neural tube. From previous research it was known that spinal cord motor neurons are generated from the ventral progenitor cells that express Nkx6.1. Based upon these results, we studied if Nkx6.1 mutant mice have defects in neuronal patterning and differentiation of spinal cord neurons. Our analysis showed that Nkx6.1 mutants have

a block in the generation of motor neurons. This block results from a dorsal-to-ventral switch in the identity of spinal cord neuronal progenitor cells. As a consequence, these progenitors differentiate into a class of more dorsal neurons instead of motor neurons.

3. Nkx6.1 Function in Neuronal Migration

In the developing CNS, Nkx6.1 is not only expressed at spinal cord levels, but is expressed throughout the entire length of the neural tube, with the exception of the prosencephalon. Therefore, we studied Nkx6.1 function in the development of neurons in the hindbrain. Contingent upon their muscle targets, hindbrain motor neurons can be subdivided into somatic, branchial, and visceral motor neurons. These different classes of motor neurons develop from distinct progenitor domains in the neural tube. In our research, we asked if Nkx6.1 differentially affects the development of these classes of hindbrain motor neurons.

We showed that Nkx6.1 mutant embryos fail to form somatic motor neurons in the hindbrain, but generate normal numbers of hindbrain visceral and branchial motor neurons. After their differentiation, certain branchial motor neurons undergo extensive migration within the hindbrain. Presently, little is known about the regulatory mechanisms that control the migratory paths of these neurons.

To study if Nkx6.1 could play a role in the control of hindbrain motor neuron migration, we tested if mutation of the Nkx6.1 gene in mice affects the migratory behavior of branchial motor neurons. To label the facial branchial motor neurons, we retrogradely injected DiI into the facial nerve of wild type and Nkx6.1 mutant embryos, and found that in Nkx6.1 mutants facial branchial motor neurons fail to migrate into their proper

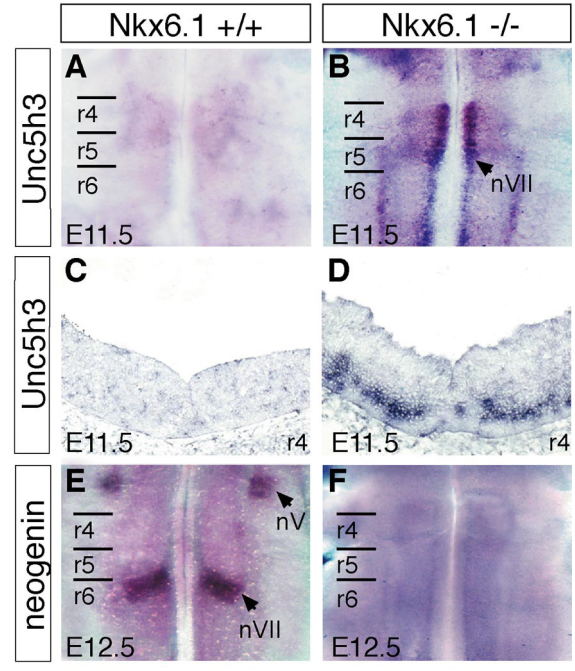

Figure 2. **Aberrant expression of the netrin receptors of *Nkx6.1* mutants.** *Neogenin* is expressed in dorsolaterally migrating facial (nVII) and trigeminal (nV) motor neurons (E). In *Nkx6.1* mutants, facial motor neurons ectopically express *Unc5h3* (B) and do not express *neogenin* (F). In situ hybridization for Unc5h3 on coronal sections through r4 verifies that the ectopic expression is specific to facial motor neurons neurons (D).

position in the hindbrain (Fig. 1). We discovered similar defects in the migratory patterns of other hindbrain branchial motor neurons. In addition, we showed that Nkx6.1 and Nkx6.2 have partially redundant roles in the migration of branchial motor neurons. While some facial branchial motor

neurons still migrate in Nkx6.1 single mutants, migration is completely stunted in Nkx6.1/Nkx6.2 double mutants.

To explore how Nkx6.1 regulates motor neuron migration, we examined the expression of numerous cell surface receptors in migrating motor neurons of wild type and Nkx6.1 mutant embryos. We found that expression of netrin receptors was altered in the absence of Nkx6.1 (Fig. 2). Given the role of netrins in neuronal migration, the aberrant expression of netrin receptors in Nkx6.1 deficient motor neurons provides a possible mechanism through which Nkx6.1 might regulate neuronal migration.

Support

Our work was supported by the Juvenile Diabetes Research Foundation, the National Institutes of Health, the Deutsche Forschungsgemeinschaft, and the Elli Lilly Forschung GmbH. Dr. Shelley Nelson was supported by the Alexander von Humboldt Stiftung, and Dr. Kirsten Kuhlbrodt by the Deutsche Forschungsgemeinschaft.

Publications

(1) Vallstedt, A., Muhr, J., Pattyn, A., Pierani, A., Mendelsohn, M., Sander, M., Jessell, T., Ericson, J. (2001) *Nkx* gene duplication and cross repression: assigning redundant and distinct roles for *Nkx6.2* and *Nkx6.1* in motor neuron and interneuron specification. *Neuron* 13: 743-755.

(2) Lioubinski, O., Müller, M., Wegner, M., Sander, M. (2003) Expression of Sox transcription factors in the developing mouse pancreas. *Developmental Dynamics* 227:402–408.

(3) Pattyn, A., Vallstedt, A., Dias, J.M., Sander, M., Ericson, J. (2003) Complementary roles for Nkx6 and Nkx2 class proteins in the establishment of motor neuron identity in the hindbrain. *Development 130: 4149-4159.*

(4) Müller, M., Jabs, N., Lorke, D., Fritzsch, B., Sander, M. (2003) Nkx6.1 controls migration and axon pathfinding of cranial branchiomotor neurons. *Development* 130: 5815-5826.

Theses

Diploma

Oelschläger, Gesa-Meike (2002). Regulation des Glukagon-Promotors durch den Transkriptionsfaktor Nkx6.1 im endokrinen Pankreas. Universität Oldenburg

Dissertations

Müller, Myriam (2003). Die Funktion des Transkriptionsfaktors Nkx6.1 bei der Entwicklung von Motoneuronen im Hirnstamm der Maus. Universität Hamburg

Henseleit, Korinna (2003). Die Funktion von Nkx6 Transkriptionsfaktoren in der Entwicklung des Pankreas der Maus. Universität Hamburg

Habilitation

Sander, Maike (2003). Die Funktion von Transkriptionsfaktoren bei der Entwicklung des endokrinen Pankreas und des zentralen Nervensystems. Universität Hamburg.

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Development and differentiation of the inner ear

Thomas Schimmang

The inner ear is induced as an auditory placode next to the developing hindbrain in vertebrates It closes to form the otic vesicle and then undergoes a complex morphogenetic process to form the mature sensory organ. We are interested in several aspects of the physiology and pathophysiology of the auditory organ, including its development and innervation, damage and degeneration of auditory neurons and hair cells and gene transfer into the inner ear. At the molecular level we are focussing on two large gene families, the neurotrophins and its receptors and the Fibroblast growth factors (FGFs). To analyse the functions of these gene families *in vivo* we are using avian (chicken) and mammalian (mice) model systems. These experiments are complemented with *in vitro* studies using cultures of hair cells and auditory sensory neurons. We perform gain-of-function (viral expression, electroporation, transgenic mice) and loss-of-function (antisense-oligonucleotides, RNAi, knock-out mice) experiments to define the roles of several members of the neurotrophin and FGF gene families during physiological and pathophysiological processes in the inner ear. Next to these studies focussing on predefined molecules we are performing a genome-wide screen to identify genes involved in various important physiological processes in the inner ear, including its development, damage processes, ageing and regeneration. This will hopefully allow us to define additional key players which act during these processes which may then be used to positively modulate pathophysiological processes in the inner ear via gene transfer.

1. The roles of neurotrophins and their receptors for innervation of the inner ear

Our studies have revealed which of the neurotrophins and their receptors, termed Trks are essential for the innervation of the inner ear in avians and mammals. Using viral gene transfer and the study of mouse mutants we could conclude that the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) acting via their high-affinity receptors TrkB and TrkC control the survival and differentiation of auditory and vestibular sensory neurons. Recently, we have analysed the consequences of a lack of the TrkB receptor and its ligand, the neurotrophin brain-derived neurotrophic factor (BDNF) in the late postnatal or adult inner ear using mouse mutants. During early postnatal development mutant animals show a lack of afferent innervation on outer hair cells in the apical part of the cochlea, whereas nerve fibres in the basal part are maintained. Strikingly, this phenotype is reversed during subsequent maturation of the cochlea which results in a normal pattern of outer hair cell innervation in the apex and loss of nerve fibres at the base in adult mutants. Measurements of auditory brain stem responses of these mice revealed a significant hearing loss. The observed innervation patterns correlate with opposing gradients of BDNF and NT-3 expression in cochlear neurons along the tonotopic axis. The reshaping of innervation thus may be controlled by autocrine signaling between neurotrophins and their receptors in cochlear neurons. Our results indicate a substantial potential for reinnervation processes in the mature cochlea which may also be of relevance for treatment of hearing loss in humans. The neurotrophins may be introduced into the inner ear via a viral system which is based on Herpes simplex virus type I (HSV-1)-mediated gene transfer. Due to the neurotropism

of HSV-1 this mode of transfer is especially suited to express genes in the peripheral nervous system, including auditory sensory neurons.

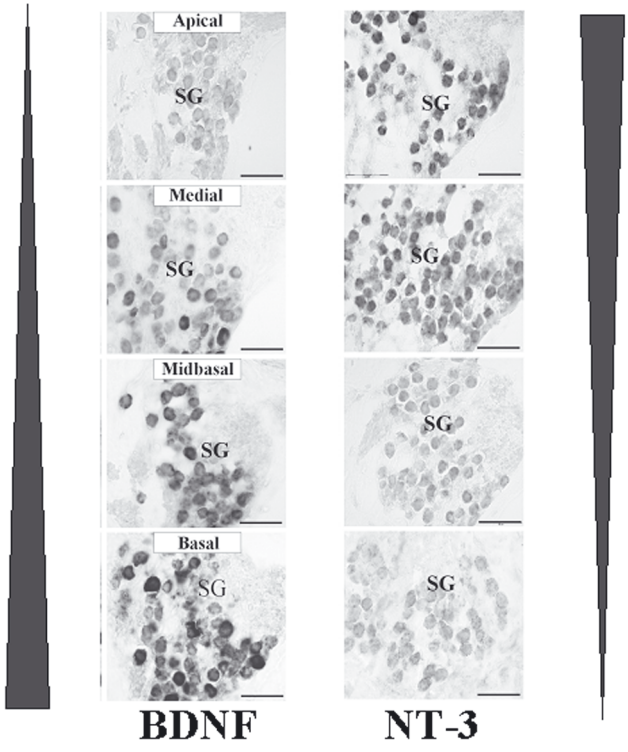

Figure 1. Neurotrophin gradients in adult cochlear neurons

2. Functions of FGF genes during inner ear development and differentiation

The FGF genes comprise a large family of 24 growth factors which have been shown to act during a vast amount of processes including development, differentiation and regeneration. Most excitingly, members of this family have been found to be responsible for the formation of organs, including lung, thymus and the brain as well as skeletal and limb development. In the inner ear, various members of the FGF gene family are expressed which suggest their involvement during the formation and differentiation of the auditory organ. We have focussed our interest on FGF3 which is expressed in the developing hindbrain next to the inner ear in many classes of vertebrates, including fish, reptiles, birds and mammals. Due to this expression pattern, FGF3 has been postulated to act as an inducer from the hindbrain which forms the otic placode in the overlying ectoderm giving rise to the inner ear. Using viral vectors we have shown that FGF3 is able to induce ectopic inner ears in avian embryos underlining its capacity as an inducer of the inner ear in the bird embryo. Our main interest is now focussed on the role of the FGF3 gene in mammals. To this end, we have carried out a series of loss- and gain-of-function experiments using transgenic mice. We generated a new mutant allele lacking the entire FGF-3 coding region but surprisingly found no evidence for defects either during inner ear development or in the mature sensory organ, suggesting the functional involvement of other FGF family members during its formation. Ectopic expression of FGF-10 in the developing hindbrain of transgenic mice lead to the formation of ectopic otic vesicles, indicating a role for FGF-10 during otic vesicle induction. Expression analysis of FGF-10 during mouse embryogenesis revealed a highly dynamic pattern in the

developing hindbrain, partially overlapping with FGF-3 expression and coinciding with formation of the inner ear. However, FGF-10 mutant mice have been reported to display only mild defects during inner ear differentiation. We thus created double mutant mice for FGF-3 and FGF-10, which form severely reduced otic vesicles, suggesting redundant roles of these FGFs, acting in combination as neural signals for otic vesicle formation.

Figure 2. FGF mouse mutant with vestibular defects

3. Gene profiling of the inner ear during development and disease

The sequencing of the human and mouse genomes has provided us with the genetic information which is necessary to set up the mammalian body plan. As a next step we need to know how this genetic information is used in space and time to fulfill the cellular functions for the development of the organism and the constant maintenance of body function during adulthood. To address these functions in the inner ear we have started to monitor the expression of genes in this sensory organ using microarrays. As a first step we have analysed the gene profile of the murine otic vesicle which is a key point during inner ear development. The use of Affymetrix gene chips has allowed us to determine the expression of 36000 genes at this developmental stage. The gene profiles determined during normal development will be compared with the profiles observed in neurotrophin receptor and FGF mouse mutants. This analysis will allow us to define target genes which are regulated by neurotrophin and FGF signalling. The definition of key target genes will also permit us to test their gene products for therapeutic purposes via gene transfer into the inner ear of mouse modells with hearing defects. On the long run these experiments thus may also lead to an amelioration of deficits in the human inner ear, which next to the eye is the most commonly affected sensory organ in our society.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft and the DAAD. Dr. Yolanda Alvarez is supported by the Spanish Ministry of Education, Culture and Sports.

Publications

(1) A. Postigo, A. M. Calella, B. Fritzsch, M. Knipper, D. Katz, A. Eilers, T. Schimmang, G. Lewin, R. Klein, and L. Minichiello. (2002) Distinct requirements for TrkB and TrkC signaling in target innervation by sensory neurons. Genes&Development 16, 633-645.

(2) E. Carnicero, M. Knipper, J. Tan, M. T. Alonso and T. Schimmang. (2002) Herpes simplex virus type 1-mediated transfer of neurorophin-3 stimulates survival of chicken auditory sensory neurons. Neurosci. Lett. 321, 149-152.

(3) M. Praetorius, M. Knipper, B. Schick, J. Tan, A. Limberger, E. Carnicero, M.T. Alonso and T. Schimmang. (2002) A novel vestibular approach for gene transfer into the inner ear. Audiol.&Neurootol. 7, 324-334.

(4) I. Vicario and T. Schimmang. (2003) Transfer of FGF-2 via HSV-1-based amplicon vector promotes efficient formation of neurons from embryonic stem cells. J. Neurosci. Meth. 15, 55-60.

(5) T. Schimmang, J. Tan, M. Müller, U. Zimmermann, K. Rohbock, I. Köpschall, A. Limberger, L. Minichiello and M. Knipper. (2003) Lack of BDNF and TrkB signaling in the postnatal cochlea leads to a spatial reshaping of innervation along the tonotopic axis and hearing loss. Development 130, 4741-4750.

(6) Y. Alvarez, M.T. Alonso, V. Vendrell, L. Zelarayan, P. Chamero, T. Theil, M. Bösl, S. Kato, M. Maconochie and T. Schimmang. (2003). Requirements for FGF-3 and FGF-10 during inner ear formation. Development 130, 6329-6338.

(7) N. Powles, C. Babbs, M. Ficker, T. Schimmang and M. Maconochie. (2004). Identification and anlysis of genes from the mouse otic vesicle and their association with developmental subprocesses through in situ hybridisation. Dev. Biol. 268, 7-23.

Thesis

Dissertation

Vendrell, Victor (2003). Induction and developmental control of the inner ear by fibroblast growth factors (FGFs) in vertebrates. Universidad de Valladolid.

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DNA Sequencing

Willi Kullmann

At the ZMNH a DNA-sequencing facility was established in October 1995. Automated DNA-sequencing started with an ABI Prism 373 DNA sequencer which was replaced by an ABI Prism 377 DNA sequencer in May 1996 to enable faster gel runs with higher throughputs. The latter was then upgraded in June 1999 from 64 to 96 gel lanes per run.

The biochemical concept underlying the above mentioned DNA-sequencers can be deduced from the chain-termination method developed by Sanger and coworkers in the late seventies. This method uses radioisotope labels in order to detect DNA-fragments, whereas the automated sequencers give preference to flourescence-based detection. Presently an improved set of fluorescence dyes (big dye) is used which greatly reduces the notorious weak G after A pattern characteristics of its predecessor.

The ABI Prism 377 sequenator enables a reading-length of about 450 bases after a gel run time of only 4 hours, whereas the number of bases which can be read after 10 hours amounts to about 750 bases.

Due to the enhanced throughput of the new sequenator, two gels can be run per day. From January 2001 until December 2003 approx . 50000 sequence analyses were performed.

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Morphology

Michaela Schweizer

As a part of the general services of the ZMNH the Morphology Unit performs investigations of neurobiological questions and supports scientists in many areas of microscopy. The histological characterisation of genetically engineered animals is becoming a major approach in our facility. We investigate the histopathology of selected organs or tissues of interest to study the effect of genetic modifications. We give advice on morphological questions, teach and train researchers in the application of microscopical techniques. We introduce and establish new techniques and guarantee efficient use of the respective equipment.

1. Offered services

- Performance of light- and electron microscopical investigations
- Advice and practical instruction in the application of histochemical techniques
- Instruction of researchers in operation of microscopes and accessories
- Introduction of useful new (immuno-) histochemical techniques and/or equipment

2. Techniques

- Morphological studies of many kinds of tissues with light-, confocal laser scanning-, or transmission electron microscopy
- Patho-histological analysis of the whole body of transgenic mice
- Histo (cyto) chemical staining procedures
- Immunohisto (cyto) chemistry
- In situ hybridisation

We prepare cell and tissue samples for scientific histological and (immuno-)histochemical light and fluorescence microscopy. All preparation steps, (including fixation, sectioning with vibratome, cryotome or microtome, staining, mounting etc.) are performed by the group. The Morphology Unit has at its disposal both conventional and fluorescence microscopes (Zeiss Axiophot), as well as two confocal scanning laser microscopes (Leica SP2) in inverted configuration.

We process cells and tissues for conventional transmission electron microscopy (Zeiss 902) and offer immunolocalisation of gene products applying pre- and postembedding protocols. We take care to preserve both, antigenicity and structural integrity.

We localise m-RNA expression routinely by hybridisation of radioactively labelled cRNA-probes to cryo-sections of fresh-frozen tissues or to cultured cells. The hybridisation signals are shown autoradiographically using high resolution X-ray-films or application of photographic emulsion followed by light microscopy.

All results are documented in high resolution digital images.

Research Group:
Functional role of the serine protease tissue plasminogen activator (tPA) in synaptic plasticity

Henrike Neuhoff, Corinna Büttgen, Michaela Schweizer

Long-term synaptic plasticity is likely to be associated with structural changes. Tissue plasminogen activator (tPA) is a serine protease that has been implicated in synaptic plasticity. Thus, tPA expression is up-regulated after induction of long-term potentiation (LTP) and tPA knock-out mice do not exhibit late-phase LTP (L-LTP). However, little is known about the localisation of tPA within neurons and hence the precise mechanism of action of tPA during plasticity. To further understand the mechanisms by which tPA might influence synaptic plasticity, we investigated the subcellular localisation of tPA by immunohistochemistry at the light and electron microscopic levels in the mouse cortex. tPA was found in a subset of presynaptic boutons of excitatory synapses but not inhibitory synapses.

A long-recognised physiological role for tPA is to convert the zymogen plasminogen into the active enzyme plasmin, which degrades various extracellular matrix molecules (Vassalli et al., 1991; Plow et al., 1995). Using double immunolabelling in combination with confocal imaging, we demonstrated colocalisation of tPA with plasminogen at single excitatory synapses, suggesting that tPA can activate the plasminogen-plasmin proteolytic cascade at the level of individual synapses. Moreover, we found tPA proteolytic activity in the extracellular medium of cultured hippocampal neurons and demonstrated that short-term tPA application to postnatal hippocampal cultured neurons induced synaptic remodelling.

These results suggest that tPA mediates structural plasticity via activation of plasminogen. Further work will study the possible interaction of tPA with other target molecules.

(1) Macias, M., Fehr, S., Dwornik, A. Sulejzak, D., Wiater, M., Czarkowa-Bauch J., Skup, M. and Schachner, M. (2002). Exercise increases mRNA levels for adhesion molecules N-CAM and L1 correlating with bDNF response. Molecular Neuroscience 13, 2527-2530

(2) Neu, A., Neuhoff, H., Trube, G., Fehr, S., Ullrich, K., Roeper, J., and Isbrandt, D. (2002) Activation of GABA(A) receptors by guanidinoacetate: a novel pathophysiological mechanism. Neurobiol Dis. 11(2):298-307.

(3) Rust, M.B., Maier, H., Seidenbecher, T., Schweizer, M., Keating, D.J., Faulhaber, J., Ehmke, H., Pfeffer, C., Scheel, O., Lemcke, B., Horst, J., Leuwer, R., Pape, H.-C., Völkl, H., Hübner, C.A., and Jentsch, T.J. (2003). Loss of K-CL co-transporter KCC3 causes deafness, neurodegeneration and reduced seizure threshold. The EMBO Journal 22, 5422-5434.

(4) Neidhardt, J., Fehr, S., Kutsche, M., Löhler, J., and Schachner, M. (2003) Tenascin-N: characterization of a novel member of the tenascin family that mediates neurite repulsion from hippocampal explants. Mol. Cell. Neurosci. 23 193-209

(5) Bukalo, O., Fentrop, N., Lee, A.Y.W., Salmen, B., Law, J.W.S., Wotjak, C.T., Schweizer, M., Dityatev, A., and Schachner, M. (2004). Conditional ablation of the neural cell adhesionmolecule reduces precision of spatial

learning, long-term potentiation, and depression in the CA1 subfield of mouse hippocampus. J. Neurosci. 24, 1565-1577.

(6) Greeve, I., Kretzschmar, D., Tschäpe, I.-A., Beyn, A., Brellinger, C., Schweizer, M., Nitsch, R., and Reifegerste, R. (2004). Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic Drosophila. J. Neurosci. (accepted).

Dissertation

Büttgen, Corinna (2003): Untersuchung der Wirkungsweise des Gewebe Plasminogen Aktivators auf die synaptische Ultrastruktur primärer hippocampaler Neurone.

Collaborations

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Prof. Dr. Guojun Bu, Washington University School of Medicine, St. Louis, USA

PD Dr. Matthias Kneussel, ZMNH

Dr. Carsten Schmidt, ZMNH

Collaborations with colleagues at the University Clinics Hamburg

Prof. Dr. Jürgen Schwarz, Institut für Physiologie

PD Dr. Patrick Friederich, Klinik für Anästhesiologie

PD Dr. Stefan Kindler, Institut für Zellbiochemie und Klinische Neurobiologie

Support

This work was supported by the DAAD and the British Council.

Structure of the Service Group

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Mass Spectrometry and Biomolecular Interaction Analysis

Christian Schulze

Each mammalian cell has numerous different proteins, and each protein has its own specific function. The function is only realized in its interaction with other molecules. The complexity of the cellular or even multicellular environment makes it neccessary to use *in vitro* assays to study the protein-protein interaction in detail. A suface plasmon resonance biosensor is available for these studies.

1. Biomolecular Interaction Analysis

Protein-protein interaction for functional characterization are performed using a surface plasmon resonance (SPR) biosensor (Biacore 3000). The technique is based on the total internal reflection phenomenon. Changes in the mass concentration of macromolecules at a biospecific interface are recorded and displayed in real time. Numerical evaluation of the data is used to derive reaction rates.

The techniques requires immobilization of one of the binding partners. This is a challenging taks for membrane proteins and proteins that require multimeric complexes for their activity. The bacterial potassium chanel KcsA is a system in which the central pore is formed by a tetramer. The active tetrameric protein complex displays remarkable stability and was immobilized using different functionalities. It retains only partial activity once solubilized and captured on the dextran sensor surface but sufficient to obtain reliable binding data (1). Subregions of KcsA were modified by substituting regions around the central pore with human Kv-channel sequences. Structure activity relations could be studied using different chimeric channel proteins and various inhibitors from scorpion venom. Experimental reaction rates and binding constants were comparable to data from filter binding assays.

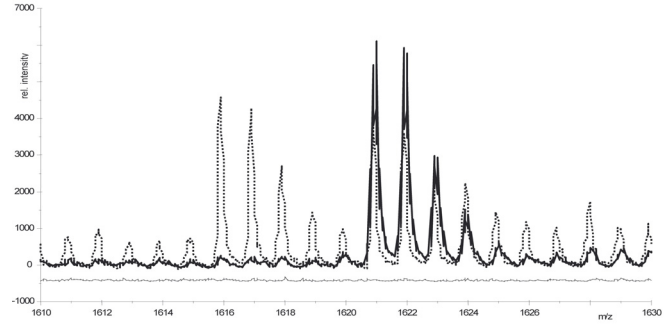

Figure 1. MALDI mass spectrum (partial) of a recovered sample digested with trypsin. Specific surface (bold), crude extract (dottet), trypsin control (lowest).

Biomolecular interaction is often complex and may not follow a straight 1:1 complex formation. This is not obvious from equilibrium binding data as they present an apparent binding constant but can be deduced from the kinetic parameters. The orphan nuclear receptor ERRγ does bind calmodulin in a Ca^{2+}-dependent fashion (2). A fit of binding data assuming a simple A + B modell shows large deviations between experimental data and fit. A two step modell in which an initial complex is allowed to undergo a conformational change toward a more stable complex can describe the data much better. Investigation of protein constructs representing

either N- or C-terminal parts of the receptor corroborate this modell. Published data for homologous proteins also indicate a more complex binding situation.

2. BIA-MS

In a conventional SPR experiment that focusses on kinetic parameters of molecule/molecule interaction purified proteins are an essential prerequisite. Often only one binding partner is available at high quality and the specific signal must be inferred using carefully selected reference surfaces. If one has to start with a complex mixture and the binding partner is unknown, the specifically bound material can even be recovered and subjected to mass spectrometric analysis for identification. The absolute amount of protein isolated with help of the integrated microfluidic system is just within the range of high sensitivity mass spectrometers. The procedure was tested with a non-fractionated mouse brain preparation. The obtained mass spectral finger print after digesting the recovered material with trypsin clearly shows the selective enrichment of one component from the starting mixture (Figure 1). This option allows one to directly work with crude cell preparations and identify even low abundant (fmol) binding partner(s) reducing both time and amount of starting material for the analysis.

Publications

(1) Legros, C., Schulze, C., Garcia, M. L. Bougis, P. E. Martin-Eauclaire, M. F., Pongs, O. (2002). Engineering-specific pharmacological binding sites for peptidyl inhibitors of potassium channels into KCSA. Biochemistry 41, 15369-15375.

(2) Hentschke, M., Schulze, C., Susens, U., Borgmeyer, U. (2003). Characterization of calmodulin binding to the orphan nuclear receptor Err gamma. Biol. Chem. 384, 473-482

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Transgenic Mouse Facility

Irm Hermans-Borgmeyer

The transgenic mouse facility supports scientists of the ZMNH and of the UKE in all aspects of production of transgenic mice.

We offer the generation of transgenic mice by pronucleus injection of DNA constructs into one cell stage mouse embryos and the injection of mouse ES cells altered by homologous recombination into mouse blastocysts. The injection laboratory is equipped with two injection set ups, one for pronucleus and one for ES cell injection.

Interested scientists and students have the possibility to watch the surgical procedures as well as the injections via a monitor.

The cell culture laboratory of the facility serves to provide ES cells and mouse embryonal fibroblasts and support to the scientists during the ES cell culture. In addition we offer help with the design of experiments, the preparation of DNA for injection and electroporation, and the analysis of recombinant ES cell clones as well as of the generated mouse lines. Furthermore, we established one ES cell line.

In 2003 fifty injection projects were performed, half of which were pronucleus injections. During the same period our ES cell culture laboratory was used to carry out seven gene targeting projects. Five of these involved the generation of conditional knock out mice. This reflects the increasing number of conditional gene targeting strategies used by the scientists of the ZMNH. Recombinant ES cell lines harboring classical “knock outs” are now often bought from different suppliers.

In parallel to the service function of the facility projects are performed in collaboration with scientists of the ZMNH to provide members of the institute with the latest development of the technologies.

Moreover, courses on transgenic techniques were held for students of medicine and biology and for animal care takers.

Publications

(1) Hermey, G., Hübner, C.A., Kuhl, D., Schaller, H.C. Hermans-Borgmeyer, I. (2004).The three sorCS genes are differentially expressed and regulated by synaptic activity. J Neurochem, in press

(2) Venero, C., Tilling, T., Hermans-Borgmeyer, I. Herrero A.I., Schachner, M, Sandi, C. (2004). Water maze learning and forebrain mRNA expression of the neural cell adhesion molecule L1. J. Neurosci. Res. 75, 17–181

(3) Stein, V., Hermans-Borgmeyer, I., Jentsch, T.J., Hübner, C.A. (2004). Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride. J. Comp. Neuro. 468, 57-64

(4) Urny, J, Hermans-Borgmeyer, I., Gercken, C., Schaller, H.C. (2003). Expression of the presenilin-like signal peptidase (SPP) in mouse adult brain and during development. Gene Expr. Patterns 3, 685–691.

(5) Ignatov, A., Lintzel, J. Hermans-Borgmeyer, I., Kreienkamp, H.J., Joost, P., Thomsen, S., Methner, A., Schaller, H.C. (2003). Role of G-protein-coupled

receptorGPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J. Neurosci., 23, 907–914.

(6) Venero, C., Tilling, T., Hermans-Borgmeyer, I.Schmidt, R., Schachner, M, Sandi, C. (2002). Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neurosci. 115, 1211-1219.

(7) Riedel I.B., Hermans-Borgmeyer I., Hübner C.A. (2002) SorLA, a member of the LDL receptor family, is expressed in the collecting duct of the murine kidney. Histochemistry and Cell Biology 118, 183-191.

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Teaching, Seminars

Teaching Sommersemester 2002 & 2003

Seminare und Vorlesungen für Mediziner und Naturwissenschaftler

ZMNH-Seminar für MedizinerInnen und NaturwissenschaftlerInnen
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Molekularbiologische Methoden in der Neurobiologie
Vorlesung
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Vorlesung und Seminar für MedizinerInnen und NaturwissenschaftlerInnen
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Projektleiter des Graduiertenkollegs „Neurale Signaltransduktion und deren pathologische Störungen“ (2 st.)

Pathologie von neurologischen Mausmutanten (Seminar)
Bartsch, Becker, Becker, Schachner Camartin (2 st.)

Morphologie und Zytoarchitektur des Nervensystems
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Neurale Signaltransduktion und deren pathologische Störungen
Sprecherin: Melitta Schachner Camartin

Teaching Wintersemester 2002/03 & 2003/04

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Ader, Bartsch, Becker, Becker, Dityatev, Loers, Kleene,
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Praktikum: Elektrophysiologische Methoden für Fortgeschrittene
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Neurobiologische Methoden für Fortgeschrittene
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Aufbaustudium Molekularbiologie

Vorlesung: Molekularbiologie I (1. Sem.)
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Neurale Signaltransduktion und deren pathologische Störungen
Sprecherin: Melitta Schachner Camartin

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Signal transduction by receptors for the repulsive axon guidance signal semaphorin 3A: the role of Rho-like GTPases

T. Moser, Göttingen
Presynaptic function of inner hair cells

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Neurotrophins and visual system

V. Haucke, Göttingen
Regulatory mechanisms in clathrin-mediated endocytosis at the synapse

T. Hökfelt, Stockholm
Neuropeptides: Superfluous or useful

M. Palacin, Barcelona
The apical reabsorption system for cystine

G. Roth, Bremen
Psychobiologische Grundlagen von Aggression und Gewalt - eine Forschungsskizze

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U. Boehm, Seattle
Tracing neural circuitry: the GnRH system

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Pax genes and islet development

M. Heisenberg, Würzburg
How the fly brain remembers odors and how it forgets them

W. A. Catterall, Seattle
Structure and regulation of calcium channels by signalling complexes

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G. Rychkow, Adelaide
Anion selectivity and gating of ClC-0 chloride channel

E. C. Cooper, Pennsylvania
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Neural to mesodermal cell type switching during Axolotl tail regeneration

S. Kröger, Mainz
Function of the synapse-organizer agrin

M. A. Schäfer, Berlin
Improving Pharma R&D Productivity by Leveraging Informatics

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A novel view on axonal outgrowth: role for phospholipid signalling

I. Mansuy, Zürich
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C. Haass, München
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H. Bading, Heidelberg
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R. Klein, Martinsried
Two-way communication via ephs and ephrins in axon guidance and plasticity

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Interactors to the cytoplasmic domain of sorLA

B. Hill, Edinburgh
Left/Right asymmetry, boundary formation and pancreatic homeosis: role of the Bapx1/Nkx3.2 gene in gut development

B. M. Riederer, Lausanne
The neuronal cytoskeleton, phosphorylation and stability of neurofilament and microtubule proteins

W. Denk, Heidelberg
Dendritic processing in the retina: how the rabbit knows which way the fox is running

A. H. Weston, Manchester
EDHF and potassium clouds: myoendothelial signalling in the vasculature

J. Offe, Atlanta
LR11 in Alzheimer's Disease and its effect on A_{β} levels

Financing

The ZMNH was financed in 2002 and 2003 by the City-state of Hamburg (FuHH), the Bundesministerium für Bildung und Forschung (BMBF) and by grants from research foundations and industry.

The FuHH funded the basic budget for the four institutes, the central facilities, the building and the junior research groups. In 2002, financing of a fourth junior research group was provided by FuHH.

In 2002 and 2003 the total budget of the Centre amounted to 11.948 and 12.097 million EUR. Presently 253 people are employed at the ZMNH (inclusive fellowships, exclusive the Institute for Cell Biochemistry and Clinical Neurobiology).

Financing by FuHH

Personnel, supplies and equipment contributed by FuHH (in thousand EUR):

	personnel costs	supplies incl. equipment
2002:	5.123	2.481
2003:	5.257	2.454

Other financing

In 2002 and 2003 members of the Centre received support from the Bundesministerium für Bildung und Forschung (BMBF) via individual project grants and research groups and from the Deutsche Forschungsgemeinschaft (DFG) via individual project grants, research groups, SFB's and graduate programs.

Further support was given by the European Community, Foundation Leducq, Philip Morris USA Inc., Christopher Reeve Paralysis Foundation, Hertie Foundation and others. Outside support amounted to 8.730 million EUR for 2002 and 2003.

The personnel and running costs provided by the various funding agencies were (in thousand EUR):

	personnel costs	supplies incl. equipment
2002:		
BMBF	608	427
DFG	827	256
EC	251	189
Foundations	408	116
Industry and other	84	64
SFB 444, 470, 545	653	461
total	2.831	1.513
2003:		
BMBF	422	447
DFG	1.170	378
EC	170	20
Foundations	330	118
Industry and other	164	59
SFB 444, 470, 545	674	434
total	2.930	1.456

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since August 2002:
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